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Inshore Records of Cetacea for Eastern Canada, 1949–68

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SERGEANT, D. E., A. W. MANSFIELD, AND B. BECK. 1970. Inshore records of Cetacea for eastern Canada, 1949–68. J. Fish. Res. Bd. Canada 27: 1903–1915.

New and previously published records are given for 19 species of Cetacea stranded naturally, netted, or shot on the coasts of eastern Canada from Cape Chidley, Labrador (60°30′N), to the United States border (45°N), from 1949 to 1968. The most commonly recorded species were *Globicephala melaena*, *Phocoena phocoena*, *Balaenoptera physalus*, and *Delphinapterus leucas*. Occurrence of *Stenella coeruleoalba* at Sable Island was confirmed. Four blue whales (*Balaenoptera musculus*) stranded naturally, three of them in the western Gulf of St. Lawrence. Groups of fin whales (*B. physalus*) were twice stranded by ice in western Newfoundland. The greatest number of species stranded at Sable Island (44°N, 60°W).

Received November 25, 1969

INTRODUCTION

TO UPDATE knowledge of the smaller Cetacea of eastern Canadian waters we used inshore records of whales and porpoises stranded naturally or killed by man except in whaling.

The area covered (Fig. 1) includes the coasts of Labrador, Newfoundland, southern Quebec, Nova Scotia, Prince Edward Island, New Brunswick, and the French-owned islands of St. Pierre and Miquelon, near the coast of southern Newfoundland. Observations from this great extent of coastline were irregular. In the 1950's they were biassed heavily towards the vicinity of marine biological stations. Although this bias remains, a reporting network has slowly been built up with help from officers of the Department of Fisheries and Forestry of Canada, from other biologists, and from press cuttings maintained for east coast newspapers since June 1966. Since 1967 A. W. Mansfield and B. Beck have visited Sable Island (43°57′N, 59°55′W) twice a year and patrolled its 50 miles of beach, finding species that have not occurred elsewhere in the region.

The time period dates from February 9, 1949, when the first full-time student of sea mammals was appointed to an east coast station of the Fisheries Research Board, and ends on December 31, 1968. Previously published papers relating to this period are: for various species in the group (Odontoceti other than the sperm whale *Physeter catodon* L.), Sergeant and Fisher (1957); and for single or a few species, Brunel (MS, 1962, MS, 1965), Cameron (1953), Dearden (1958), Fisher and Sergeant (1953), and Sergeant (1958, 1963). For nomenclature we follow Rice and Scheffer (1968) and for suprageneric systematic order, Fraser and Purves (1960).

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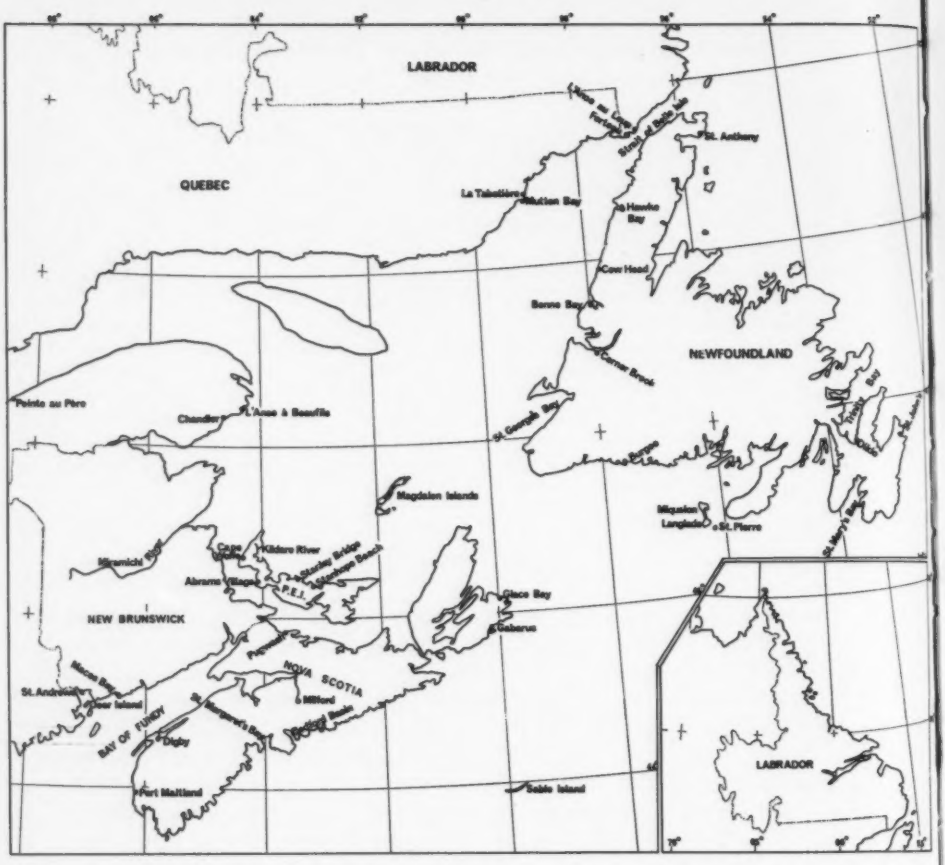

FIG. 1. Eastern Canadian waters, showing most place names mentioned in text.

MYSTICETI

FAMILY BALAENIDAE

Balaena glacialis Müller. Black right whale.

1. One specimen, alive, at Pugwash, N.S. (45°52′N, 63°40′W), October 1954. Length estimated from photograph (Fig. 2) at 35–40 ft (11–12 m). Identified by Dr H. D. Fisher from interview with finder and photographs.

FAMILY BALAENOPTERIDAE

Balaenoptera acutorostrata Lacépède. Minke whale, lesser rorqual.

1. One specimen, alive, at Digby, N.S. (44°40′N, 65°50′W), April 23, 1967. Length reported as 16 ft (4.5 m). Identified by white upper surface of flipper shown in photograph in Digby *Courier*, April 27, 1967.

Three previous inshore records (Sergeant, 1963, p. 1503): one specimen temporarily caught in an inlet behind seal net and one female 27 ft (8.2 m) long shot at Nain, Labrador (56°33′N,

FIG. 2. *Balaena glacialis* at Pugwash, 1954. Photos courtesy Dr H. D. Fisher.

61°43′W) in December 1961; and a young immature animal caught in a herring weir, from the New Brunswick side of the Bay of Fundy on August 4, 1953.

This is the first authenticated stranding of this species in the area.

Balaenoptera physalus L. Fin whale.

1. One specimen, dead, at Stanhope Beach, northern coast of Prince Edward Island (46°25′ N, 63°05′W), October 25, 1958. Length reported in local newspaper as both 45 and 56 ft (14 and 17 m). Identified from photographs in unidentified newspaper and forwarded by J. A. McLean.

2. About 12 specimens, dead, at St. George's Bay, west coast of Newfoundland, about March 1959; evidently caught in ice. Length estimated at 50 ft (15 m) from photograph, taken by resident, showing man standing on carcass. Identified by staff of whaling station at Dildo, Trinity Bay, where whales were taken.

3. One specimen, probably of this species, dead, on west side of isthmus joining Petite Miquelon and Miquelon islands (46°55′N, 56°20′W), July 20, 1960. Length 72 ft (22 m). Measured by the author–naturalist the late Henry Collins, New York, who gave the following details to Dr J. C. Moore: white baleen, blackish skin dorsally, and 59 ventral grooves.

4. One specimen, dead, at Etang des Caps, Magdalen Islands, Que. (47°15′N, 62°00′W), about November 15, 1962. Length 64–65 ft (19.5 m). Identified from length and other evidence received from L. Rowdon, Marine Biological Station, Grindstone, Magdalen Islands — general colour blue-black, with white and yellow underparts; half-girth, 16 ft (4.9 m); fore flipper, 7 ft × 2 ft (2.1 × 0.6 m); length from tip of flipper to tip of jaw, 12 ft (3.65 m) — and from agreement of baleen plate in colour and form with that of an immature female in collection of Fisheries Research Board of Canada's Arctic Biological Station.

5. One specimen, alive, at Burgeo, Nfld. (47°37′N, 57°36′W), January 21, 1967. Died of gunshot wounds about February 3. Identified by F. Mowat as a female, length about 65 ft (20 m). Had entered a lagoon 500 × 300 ×15 m deep, probably in search of herring; a companion was reported to have remained outside for several days.

6. At least 12 rorquals, probably fin whales, caught in ice in northern Gulf of St. Lawrence and Strait of Belle Isle between March 8 and 28 or later observed dead; ice known to have formed on northwest coast of Newfoundland between late February and mid-March and lasted till early April (Canada, Department of Transport, unpublished ice charts). Details as follows.

A live whale trapped in western arm of Bonne Bay, near Woody Point (49°36′N, 57°57′W), from March 8 to at least March 12 (St. John's *Evening Telegram*, March 13) probably among the first strandings.

One dead whale floating off Port au Choix, Nfld. (50°42′N, 57°25′W) on March 28 estimated to be 60 ft long. Identified as a fin whale by Fishery Officer E. M. MacKay.

Four individuals, dead, at Forteau and Fox Cove, Labrador (51°23′N, 57°00′W), three at Hawke Bay (50°40′N, 57°20′W) and 8–10 between South Head, Bonne Bay (49°33′N, 58°00′W),

and Cow Head (49°56′N, 57°49′W), Nfld., before April 22 estimated as 40–70 ft (12–21 m) long (H. V. E. Smith, Department of Fisheries and Forestry of Canada, and Dr W. Templeman, Fisheries Research Board of Canada, St. John's, Nfld.).

Many dead whales seen floating near La Tabatière and Mutton Bay, Que. (51°N, 59°W), on June 3 (J. Graham, Department of Fisheries and Forestry of Canada, Quebec).

The points to which the dead whales were carried (Fig. 3) can be explained by the known patterns of surface drift (Huntsman et al., 1954).

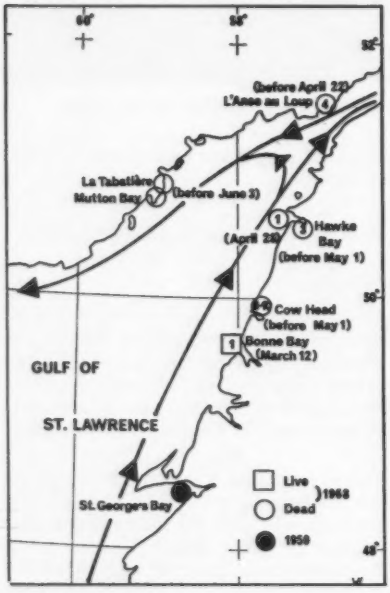

FIG. 3. Strandings of probable fin whales in Gulf of St. Lawrence, 1959 and 1968. Numerals show numbers of animals when known. Arrows show direction of surface current drift (Huntsman et al., 1954).

7. Three skulls of large Mysticeti were found washed up on Sable Island in recent years. One such skull was sketched by Mansfield on January 27, 1968. The greatest width was measured at 176 cm. It appears to belong to a large *Balaenoptera* and by comparison with the measurements in True (1904, table p. 133) might come from a fin whale about 50 ft (15 m) in length.

In sum there were two multiple strandings, both caused by ice and presumably referring to fin whales, and at least four single strandings of fin whales.

Balaenoptera musculus L. Blue whale.

1. One specimen, probably of this species, dead on north beach of Sable Island, February 1958. Examined by G. McAlpine, Canada Department of Transport. Identified by Dr H. D. Fisher and Dr A. W. Mansfield from length given as 80–90 ft (24–27 m), and from light grey ventral grooves seen in colour transparency.

2. One specimen, dead, at Pointe-au-Père, Que., on south shore of St. Lawrence River (48°31′N, 68°28′W) on August 20, 1962, and later at Baie des Sables a few miles east. Identified as a blue whale from length given as 75 ft (23 m) and from grey belly, seen in photographs (*Montreal Gazette*, September 21, 1962; Mont Joli Police Station) by D. E. Sergeant and by G. Williamson, familiar with the species in the Antarctic.

Two previous records: one specimen, female, dead, at Pointe-au-Père, Que., early in October 1951 (Cameron, 1953); length estimated at 82 ft (25 m) and circumference at 35 ft (10.7 m); identified by Abbé E. Talbot; account and photograph in newspaper *Progrès du Golfe*, October 5, 1951. One female, dead, at L'Anse-au-Beaufils, Gaspé-Sud (48°29′N, 64°19′W) on June 5, 1964 (Brunel, MS, 1965, p. 65); length 75 ft (22 m); identified from size and from colour of baleen (black), belly (streaked with grey or black in which some blue evident), and flipper (white ventrally and black dorsally).

In sum there are four records of blue whales for the 20-year period, of which three come from the western Gulf of St. Lawrence and one from Sable Island.

Megaptera novaeangliae Borowski. Humpback whale.

One previous record: a specimen 50 ft (15 m) long, stranded at Chandler, Gaspé-Sud (48° 22′N, 64°38′W) on November 30, 1961 (Brunel, MS, 1962, p. 150). Several specimens of the barnacle *Coronula diadema* (L) and the amphipod parasite *Cyamus boopis* Lütken collected from the ventral surface by Brunel.

ODONTOCETI

Family Ziphiidae

Hyperoodon ampullatus Forster. Bottlenose whale.

1. One specimen (Table 1), male, found on south beach of Sable Island by A. W. Mansfield and B. Beck on January 12, 1968. Since the species is hunted by small whale-catchers from Nova Scotia in the area of the Gully, northeast of Sable Island, the carcass may have been that of a killed animal.

Sergeant and Fisher (1957) recorded a specimen probably of this species taken by the whaling station at Dildo, Nfld., on July 27, 1953, and cited one previous record from Cap Martin in the estuary of the St. Lawrence River, Que., in 1940.

Mesoplodon bidens Sowerby. North Sea beaked whale.

No further examples have appeared since the two from Newfoundland included in Sergeant and Fisher (1957).

Mesoplodon densirostris Blainville. Dense-beaked whale.

1. One specimen washed ashore at Belfrey Beach, Fourchu Bay, Cape Breton Island, N.S. (45°50′N, 60°10′W), in December 1968. Estimated length 18–20 ft (5–5.6 m); described by finder as blue-black on dorsal side and white on belly. Tooth and part of left ramus of mandible enclosing tooth preserved in formalin by finder and given to Beck, together with drawing of head, which clearly identified the animal as a *Mesoplodon*. Tooth (Fig. 4) 143 mm in maximal height, 79 mm in maximal length, 40 mm in maximal thickness. Identified as an adult male *M. densirostris* from great depth of preserved mandibular fragment, orientation of tooth in fragment, and great length of tooth at gumline as compared with the small thickness.

This is the second record of *M. densirostris* for Nova Scotia and the most northerly of eight now known from the eastern North American seaboard, including the Bahamas (see Moore, 1966).

Family Monodontidae

Delphinapterus leucas (Pallas). White whale, beluga.

1. One specimen captured in Bedford Basin, Halifax, N.S. (44°41′N, 63°38′W), late May 1968. Length 8.5 ft (260 cm), colour light grey, sex unknown. Identification by the late Dr A.

Table 1. Body measurements (cm) of Cetacea from Sable Island.

	Hyperoodon ampullatus (♂)	*Kogia breviceps*	*Phocoena phocoena* (PP1, ♀)	*Globicephala melaena* (♀) (GM2)	*Lagenorhynchus acutus* (LA1, ♀)	(LA2)	(LA3)	*Stenella coeruleoalba* (Dolphin 1)	(Dolphin 2)	*?Stenella* (Dolphin 3, ♂)
Date	Jan. 12, 1968	Jan. 29, 1969	Jan. 16, 1968	Jan. 14, 1969	Jan. 12, 1968	Feb. 16, 1969	Feb. 16, 1969	Jan. 12, 1968	Jan. 12, 1968	Jan. 27, 1968
No. teeth visible	L 8 $\frac{0}{1}$	L $\frac{?}{14}$	$\frac{25}{?}$	None	R $\frac{0}{1}$	$\frac{38}{?}$	–	L $\frac{42}{42}$ R $\frac{40}{42}$	$\frac{?}{43}$	37–40
1. Snout to notch of flukes	870	240	130	201	188	186	246	175	213	219
2. Snout to tip of dorsal fin	615	140	–	109	105	–	–	106	117	125
3. Snout to insertion of flipper	235	–	–	38	–	–	–	–	48	–
4. Snout to ear	–	–	–	29.5	–	–	–	–	–	–
5. Snout to centre of blowhole	145	27	–	25	26	26	30	33	34	37
6. Snout to centre of eye	–	34	–	21	26	26	30	32	34	34
7. Snout to gape	–	–	–	17	22	–	–	–	–	–
8. Snout to base of beak	–	–	–	2	4	–	–	–	12.5	15
9. Notch of flukes to genital aperture	–	–	–	79	–	–	–	–	–	77
10. Notch of fluke to anus	–	–	–	71	–	–	–	–	1	–
11. Projection of lower or upper jaw	–	–	–	1	0.5	–	–	–	–	–
12. Girth behind flippers	–	–	–	101	–	–	–	–	–	–
13. Anterior length of flipper	104	30	21	41	29	30	36	26	30	34
14. Axillary length of flipper	77	26	14	32	20	20	26	–	21	24
15. Max width of flipper	28	12	5	10.5	16.5	11	14	8	9.5	10
16. Length of base of dorsal fin	80	20	19	35	33	26	40	23	24	27
17. Height of dorsal fin	–	9	8	14	19	18	25	15	16	17
18. Span of flukes	–	56	29	38	47	40	53	30	43	40
19. Width of flukes from anterior curve to notch	–	21	10	12	15	13	16	–	13	11

H. Leim (Fisheries Research Board of Canada, 1959), confirmed by D. E. Sergeant from photograph taken by R. M. Brackett, Halifax *Mail-Star*.

2. One specimen from Kildare River, P.E.I. (46°50′N, 64°03′W), about May 1960. Animal captured with a net on July 5 and transported alive to New York Aquarium, where it soon died. An adult female, white in colour, length 10 ft (315 cm) and weight 1800 lb (860 kg). Details supplied by Dr A. H. Leim from Saint John *Telegraph-Journal*, July 13, 1960, and Dr C. Ray, New York, N.Y.

3. One specimen killed at Fox Point, St. Margaret's Bay, N.S. (44°40′N, 64°W), early May 1965. Length estimated at 15 ft (4.5 m), weight at 1600 lb (730 kg). Identified by Mr P. F. Brodie, Nova Scotia Museum of Science, who saw specimen and interviewed finder.

4. One specimen, washed ashore at Maximville, P.E.I. (46°24′N, 64°08′W), May 25, 1967. Length 14 ft 6 in (440 cm), upper teeth 8, lower 9 on each side and considerably worn, suggesting advanced age. Identified by M. L. H. Thomas, who took colour photographs from which identification was confirmed by D. E. Sergeant.

5. One specimen, probably of this species, caught in a seal net at Goose Cove, St. Anthony district, Nfld. (51°18′N, 55°38′W), early 1968. Length about 14 ft (4.3 m). Described by finder as being without beak or dorsal fin, having teeth in both jaws, and colour grey-brown. Identified by finder and by Fishery Officer S. W. LeDrew from illustration in Fraser's (1966) guide.

Additional published extralimital records are as follows: Fisher and Sergeant (1954) saw a specimen that had been captured at Maces Bay, N.B., in the Bay of Fundy on June 12, 1952. South of the Canada–U.S. border, Mairs and Scattergood (1958) recorded one in the Penobscot River at Bangor, Maine, on April 30, 1954. All these records are figured in Sergeant and Brodie (1969).

The total is four certain and one probable new record of extralimital white whales, two from Nova Scotia, two from Prince Edward Island, and one from Newfoundland. All are strandings of individuals.

Vladykov (1944) described the migrations of the white whale population inhabiting the estuary of the St. Lawrence River. Normal distribution in spring extends to Baie des Chaleurs in the southern Gulf of St. Lawrence (Vladykov, 1944, fig. 48). It seems likely from the records cited above that occasional white whales follow the Gaspé current, which flows east along the coast of the southern Gulf of St. Lawrence. They may then continue round Cape Breton Island and south along the Nova Scotia coast, and finally turn north into the Bay of Fundy. These currents carry cool water of low salinity (Hachey et al., 1954).

The north Newfoundland specimen, if indeed a beluga, most probably came from one of the arctic populations of this species, described by Sergeant and Brodie (1969).

FAMILY PHYSETERIDAE

Kogia breviceps Blainville. Pigmy sperm whale.

1. One decayed specimen (Table 1) at Sable Island, January 1969. Whole skeleton recovered by B. Beck.

Mandibular symphysis some 80 mm long and ventrally keeled. Left mandible with 14 teeth, all having open roots. Identified as *K. breviceps* from characteristics of mandibular symphysis and number of teeth (Handley, 1966), and as half-grown example from open roots of teeth together with body length.

One previous record from Nova Scotia, as noted by Sergeant and Fisher (1957).

Physeter catodon L. Sperm whale.

1. One specimen, dead, on north beach of east spit of Sable Island, February 6, 1964. Stranded too far out to be accessible but identified by A. W. Mansfield and B. Beck from size and head form.

FAMILY PHOCOENIDAE

Phocoena phocoena L. Harbour porpoise.

1. One specimen from Hardy's Channel, Prince Edward Island (46°40′N, 63°52′W), August 15, 1964. Kept frozen at Biological Substation of Fisheries Research Board of Canada, Ellerslie, P.E.I. by M. L. H. Thomas and examined by D. E. Sergeant.

2. One specimen caught in sardine weir at St. Andrews, N.B. (45°04′N, 67°03′W), August 10, 1965, and shot. Carcass identified by Dr J. C. Medcof, Biological Station, St. Andrews.

3. Four animals caught alive in weir between Deer and Pendleton islands, N.B. (45°02′N, 66°56′W), between August 10 and September 8, 1965, and held alive for transport to New York Aquarium. Information provided by Dr J. C. Medcof, St. Andrews, N.B., and Dr C. Ray, New York.

4. One fresh carcass (Table 1) found by A. W. Mansfield and B. Beck on Sable Island, January 16, 1968.

5. Two specimens captured at Quidi Vidi, St. John's, Nfld. (47°31′N, 52°39′W). One a female, weight 110 lb (50 kg); the other a juvenile, weight 40 lb (18 kg). Information and photograph in St. John's *Evening Telegram*, July 22, 1968.

6. One carcass at Cavendish beach, P.E.I. (46°31′N, 63°22′W), July 1968. Found by D. E. Sergeant.

These five strandings and five captures must represent a small fraction of the inshore records of harbour porpoises in the area. They fit the picture obtained from previous descriptions by Gilpin (1878), Sergeant and Fisher (1957), and Neave and Wright (1968), which suggest maximum abundance in the Bay of Fundy, moderate abundance in the Gulf of St. Lawrence, and relative scarcity in north and northeast Newfoundland. However, Williamson (1963) recorded a new-born harbour porpoise from the stomach of a Greenland shark, *Somniosus microcephalus* (Bloch and Schneider), on the eastern edge of the Grand Bank at 46°33′N, 47°23′W on June 11, 1962, where the free-living animal has not been observed. The locality, though too cold for parturition at 1.3–3.2 C surface temperature, lay close to warmer surface temperatures eastward. The wintering animal at Sable Island came from a similar position.

FAMILY DELPHINIDAE

Orcinus orca L. Killer whale.

1. One specimen, dead, at Green Island Cove, Strait of Belle Isle (51°22′N, 56°34′W), late February 1960. Identification based on reported length of 24 ft (7 m), presence of teeth in both jaws, and a seal in the stomach. Strait of Belle Isle open at the time. Information sent from Mr H. C. R. Keen, Corner Brook, to Dr W. Templeman.

2. One specimen, dead, at Cape Wolfe, P.E.I. (46°53′N, 64°14′W), June 25, 1968. Probably a female; 19 ft 11 inches (607 cm) long. Identified from colour slides of ventral surface by characteristic colour pattern. Measurements and photographs sent to D. E. Sergeant by M. L. H. Thomas.

These two new records, one certain and one probable, add to the mass stranding in Trinity Bay, Nfld., described by Dearden (1958) and a single

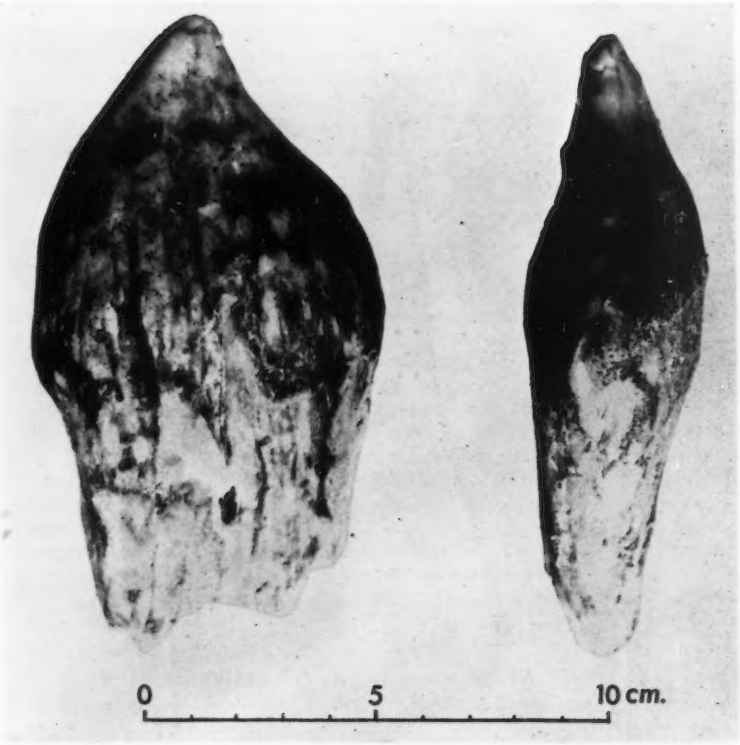

FIG. 4. Internal (*left*) and anterior (*right*) views of left mandibular tooth of *Mesoplodon densirostris*.

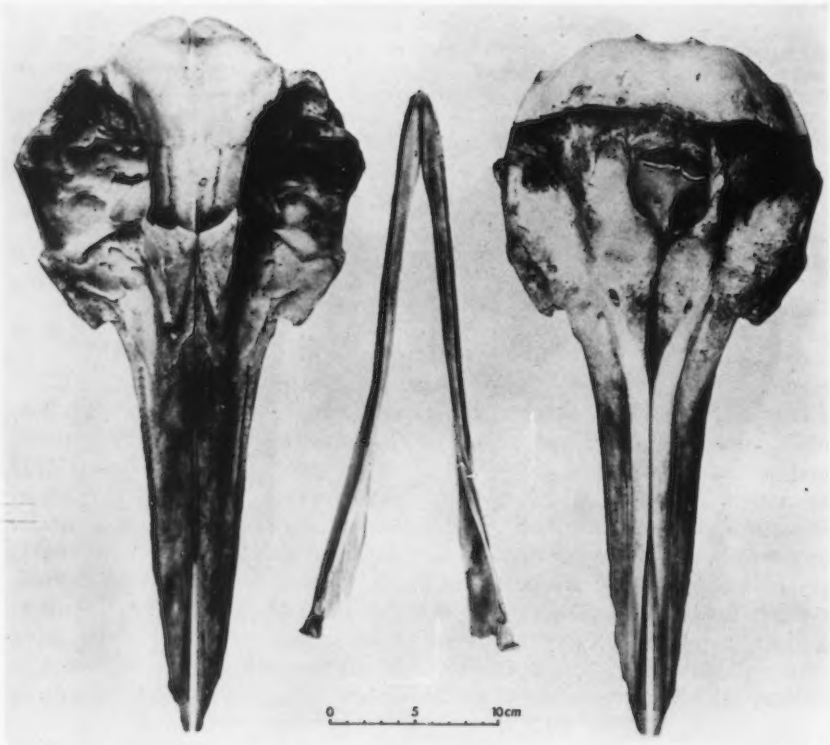

FIG. 5. *Stenella coeruleoalba*. Ventral (*left*) and dorsal (*right*) views of skull and dorsal (*centre*) view of mandibles of Dolphin 1, Sable Island, 1968.

stranding in the Bay of Fundy during the study period, as described by Sergeant and Fisher (1957, table III).

Globicephala melaena (Traill). Pilot whale, blackfish, pothead whale.

1. Twelve or more specimens stranded at Cow Head, Nfld. (49°51′N, 57°48′W), before August 4, 1957. Examined by D. Cant, Corner Brook, on that day, and identified by D. E. Sergeant from colour slides fowarded by Dr W. Templeman. One animal only evidently of calf size.

2. Eighteen stranded alive on north beach of Sable Island October 2, 1959, but refloated themselves on a later tide. Identified by Dr H. D. Fisher and A. W. Mansfield from colour photographs by G. McAlpine, Canada Department of Transport.

3. Fifty-eight, dead, at Port Maitland near Yarmouth, N.S. (44°N, 66°10′W), on night of August 1–2, 1960. Of 53 sexed animals, 32 female and 21 male. Details sent to D. E. Sergeant by E. G. Sollows, Biological Station, St. Andrews. Identification confirmed from photographs taken by K. Oxner, Yarmouth.

4. One specimen, alive, temporarily stranded in Miramichi River near Chatham, N.B. (47°01′N, 65°30′W), on or about October 1, 1961. Identified as large male of this species from length of 18 ft (5.5 m) and photograph in Toronto *Daily Star*, October 3, 1961, showing the thick dorsal fin. It eventually refloated and swam off.

5. Fifteen, alive, at Glace Bay, Cape Breton Island, N.S. (46°13′N, 59°57′W), December 24, 1967. Reported in the Halifax *Chronicle-Herald* December 27, 1967. One female measured at 14 ft (426 cm) and identified by W. Hoek, Fisheries Research Board of Canada, Arctic Biological Station, on January 20, 1968, by which time most carcasses had washed to sea.

6. One specimen (Table 1) dead, on south beach of Sable Island, February 1968. Length 17 ft (520 cm) and flippers very long, suggesting an adult male. Examined by A. W. Mansfield and B. Beck in June 1968.

7. One female caught in a fishing net at Stanley Bridge, P.E.I. (46°31′N, 63°29′W), about 1966. Colour postcard seen by D. E. Sergeant on sale at site of capture, July 1968. Length of about 9 ft (3 m) indicated by comparison with men in picture. Date of capture recalled by finder.

8. One female, dead, on east spit of Sable Island, January 14, 1969. Length 201 cm (6 ft 7 in). Determined as newborn animal from absence of teeth and birth creases on side of body. Examined by B. Beck.

One previous record: one specimen, alive, at L'Anse-au-Beaufils, Que. (48°28′N, 64°19′W), July 29, 1961 (Brunel, MS, 1962, p. 150). Length measured as 6 ft (1.8 m).

Four additional carcasses identified by B. Beck on Sable Island in January of 1967–69 but not closely examined.

We have therefore eight new, and one previous, documented records of *G. melaena*: four from Nova Scotia, including three from Sable Island, two from Quebec, and one each from New Brunswick, Newfoundland, and Prince Edward Island. Of these, four were multiple strandings and five were single strandings, giving a total of at least 109 individuals. Five records came from the months between July and October, one in December, one in February, one in late fall or winter, and one was undated.

Lagenorhynchus acutus Gray. White-sided dolphin.

1. One specimen caught by a fisherman at Mal Bay, St. Mary's Bay, Nfld. (46°57′N, 53°38′W), June 28, 1967. Examined and photographed by M. C. Mercer, Fisheries Research Board of Canada, Biological Station, St. John's.

2. One female (Table 1), dead, on south beach of Sable Island, January 12, 1968. Length 188 cm. Identified by A. W. Mansfield and B. Beck by colour pattern and preserved skull.

3. Two single specimens, dead, at Sable Island, February 1969. Both identified by B. Beck from body measurements (LA2 and LA3 of Table 1) and from collected skulls.

Sergeant and Fisher (1957) recorded two specimens driven ashore together with pilot whales at Chapel Arm, Trinity Bay, Nfld., on July 30, 1954.

Lagenorhynchus albirostris Gray. White-beaked dolphin.

No new records are reported here. Sergeant and Fisher (1957) omitted reference to the paper of Gilpin (1878) who described a contemporary porpoise fishery by Micmac Indians in the Bay of Fundy and recorded two *L. albirostris* shot at Digby Gut in August 1875. One specimen measured 5 ft 10 in (178 cm) in length; the other was larger. Gilpin reported several skulls in the Provincial Museum, Halifax, from the Bay of Fundy, Sable Island, and Halifax Harbour, but we have not been able to trace them.

Tursiops truncatus L. Bottlenose porpoise.

1. A probable specimen, dead, at Milford, N.S. (45°05′N, 63°25′W), September 3, 1968. Estimated length 6–7 ft (1.8–2.1 m) and weight 600 lb (270 kg). Identified by finder from illustrations in Fraser's (1966) guide. Carcass washed away 2 days after stranding and no specimens or photographs available to confirm identification, possible also as a large *Lagenorhynchus* (e.g., *L. albirostris*). Locality at upstream limit of tidal penetration from Bay of Fundy into Shubenacadie River.

Sergeant and Fisher (1957) recorded one specimen from New Brunswick.

Stenella coeruleoalba Meyen. Striped or euphrosyne dolphin.

1. Three living specimens on north beach of Sable Island, summer 1964. Identified by A. W. Mansfield and B. Beck in January 1968 from several black and white photographs obtained from F. Androschuk, Canada Department of Transport, officer in charge, Sable Island.

2. Three single specimens at Sable Island, January 12 and 27, 1968. One fresh carcass and two older ones (Dolphins 1, 2, and 3 in Table 1). Identification by authors confirmed by Dr E. D. Mitchell: first two specimens from skulls (Fig. 5), and third presumed to be of same species from earbones after buried skull lost; second also from colour pattern.

Mansfield (1967) provided the first record of this species from the Canadian Atlantic seaboard by examining and sketching a dead animal at Sable Island on February 14, 1967, but was unable to bring out skeletal material. The additional three specimens confirm his identification. The occurrence of *S. coeruleoalba* so far only at Sable Island suggests that it is normally confined to Gulf Stream or continental slope[1] waters.

Delphinus delphis L. Common or saddleback dolphin.

There are no recent inshore records though the species is seen very commonly at sea off Nova Scotia (Sergeant and Fisher, 1957) and southern Newfoundland. Sergeant (1958) recorded a male, length 190 cm, shot in inshore waters of Trinity Bay (47°32′N, 53°33′W) on July 22, 1957.

DISCUSSION

Nine species are recorded from Sable Island, eight from mainland Nova Scotia and Newfoundland, six from Quebec, five each from New Brunswick and Prince Edward Island, and three from Labrador (Table 2). The sampling was probably more intensive along inhabited than uninhabited coasts. Nevertheless, the overall bias is probably low, so that the waters around Sable

[1]There are three skulls identified by H. Piers as from dolphins of this genus, one of them tentatively as from this species, in the Nova Scotia Museum of Sciences at Halifax, but these bear no labels giving locality and may even have been collected at sea.

TABLE 2. Records of Cetacea stranded on the east coast of Canada, 1949–68.

Species	This report		Previously published		Total	
	No. times recorded	Total no. individuals	No. times recorded	Total no. individuals	No. times recorded	Total no. individuals
Balaenoptera acutorostrata	1	1	2	3[a]	3	4
B. musculus	2	2	2	2[b,c]	4	4
B. physalus	6	ca. 25	–	–	6	ca. 25
Delphinapterus leucas	5	5	1	1[d]	6	6
Delphinus delphis	–	–	1	1[e]	–	–
Eubalaena glacialis	1	1	–	–	1	1
Globicephala melaena	9	108	1	1[b,f]	10	109
Hyperoodon ampullatus	1	1	–	–	1	1
Kogia breviceps	1	–	–	–	1	1
Lagenorhynchus acutus	4	4	1	2[f]	5	6
L. albirostris	–	–	2	8[f]	2	8
Megaptera novaeangliae	–	–	1	1[b]	1	1
Mesoplodon bidens	–	–	2	2i	2	2
M. densirostris	1	1	–	–	1	1
Orcinus orca	2	2	2	26[f,g]	4	28
Phocoena phocoena	10	10	1	1[f]	11	11
Physeter catodon	1	1	–	–	1	1
Stenella coeruleoalba	4	6	–	–	4	6
Tursiops truncatus	–	–	1	1[f]	1	1

[a–g]References: [a]Sergeant, 1963; [b]Brunel, 1962, 1965; [c]Cameron, 1953; [d]Fisher and Sergeant, 1953; [e]Sergeant, 1958; [f]Sergeant and Fisher, 1957; [g]Dearden, 1958.

Island evidently have the most diverse cetacean fauna of the region and this fauna becomes less varied on mainland coasts and also as one proceeds northward. This difference could be accounted for by the proximity of Sable Island to deep water, by its situation in the region of mixing of the "slope water" between Gulf Stream and coastal water (McLellan, 1954), and by the lower species diversity in colder waters of the Labrador Current.

Since *L. acutus* stranded more frequently at Sable Island than inshore, and *S. coeruleoalba* only at this locality, we regard them as offshore species. In contrast *P. phocoena* stranded more commonly on mainland coasts than at Sable Island and is therefore an inshore species. *Globicephala melaena* stranded indifferently at Sable Island and the mainland. *Delphinus delphis* stranded less frequently anywhere than one would expect from the frequent sightings of the species.

Comparison with strandings on British coasts (Fraser, 1953, p. 15) shows that *B. musculus*, *B. physalus*, *D. leucas*, and *G. melaena* strand more commonly in eastern Canada than in Britain; and *T. truncatus*, *D. delphis*, and *Grampus griseus* much more commonly in Great Britain. The remaining species strand about equally where there are sufficient records to enable a comparison to be made. Although *T. truncatus* and probably also *G. griseus* scarcely reach waters off eastern Canada, the absence of *D. delphis* from eastern Canadian strandings

is difficult to explain. *Balaenoptera musculus* and *B. physalus* have been hunted to lower levels off European coasts, explaining their rarer stranding in Britain.

We recognise three types of natural strandings. First, through accident, disease, or other cause a whale may float or swim ashore dead or dying. Second, an individual or herd may become trapped by a lagoon or sandbar. In the study region, as elsewhere, pilot whales (*G. melaena*) are prone to this hazard and the trapping of a fin whale in a lagoon is recorded above. A third category, unique to northern and southern seas, is important in the study area: a herd of Cetacea may be forced by advancing sea ice onto a lee shore. Two recent examples and one historic example are cited here, all probably referring to fin whales (*B. physalus*). All three refer also to the west coast of Newfoundland, which remains open until late in the winter since the Coriolis force bends to the right the warm currents entering the Gulf of St. Lawrence. Prevailing westerly winds in late winter may then close off the open water, trapping the whales. However, two previously published examples of ice-trapping, referring to white-beaked dolphins (*L. albirostris*) (Sergeant and Fisher, 1957) and killer whales (*O. orca*) (Dearden, 1958), occurred in eastern Newfoundland.

Here, too, single examples of large whales, probably fin whales, have been reported in spring caught in ice but escaping (e.g., St. John's *Evening Telegram*, April 1953). There are also the late-season records of minke whales at Nain, Labrador, cited above. Clearly all these species must be cold-adapted in order to have remained until ice moves in. The sub-antarctic southern bottlenose whale *Berardius arnouxi* Duvernoy, together with *B. acutorostrata* and *O. orca*, has also been reported trapped by ice (Taylor, 1957). The true arctic whales such as *D. leucas* are able to break ice with their backs, and though they sometimes become trapped by ice may survive the episode unless killed by man (Freeman, 1968).

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Certain Aspects of Production and Standing Stock of Particulate Matter in the Surface Waters of the Northwest Atlantic Ocean[1]

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SUTCLIFFE, W. H., JR., R. W. SHELDON, AND A. PRAKASH. 1970. Certain aspects of production and standing stock of particulate matter in the surface waters of the northwest Atlantic Ocean. J. Fish. Res. Bd. Canada 27: 1917–1926.

Samples collected during January and October 1968 from 44° to 19°N in the western North Atlantic were analysed for particulate organic carbon, ATP, DNA, chlorophyll *a*, and total particulate volume between 1 and 40 μ diameter. Although the ratio of chlorophyll to ATP decreased from north to south, the ratio of particulate carbon to ATP did not show any systematic trend. A good correlation was found between ATP and DNA even though the DNA concentration was too great to be entirely representative of living material. Growth or production of particulate material as measured by the Coulter Counter method was higher than that measured by C-14 method. A trend was noted towards higher specific growth rate with smaller standing crop in southern waters.

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INTRODUCTION

SEVERAL METHODS have been proposed for estimating the living fraction (mostly algal) of the suspended particulate matter in the sea. Of these, the chlorophyll method has been the most widely used. However, chlorophyll is subject to variation with a number of factors as indicated by Eppley (1968), who also raised objections to general application of the chlorophyll:carbon ratio method proposed by Steele and Baird (1961).

Cushing and Nicholson (1966) and Cushing et al. (1968) developed a method based on a series of growth measurements of incubated material by Coulter Counter. Another incubation method using carbon assimilation and chlorophyll was given by Eppley (1968). There are, however, two disadvantages to the estimation of biomass by growth measurements: the results are indirect, and the effect of long confinement (2–3 days) in bottles is not well understood.

There appears to be merit in estimation of biomass by measurement of adenosine triphosphate (ATP) proposed by Holm-Hansen and Booth (1966) and Holm-Hansen (1969). This has the advantage of giving a single, direct, and "instantaneous" measure of living material. Likewise, deoxyribonucleic acid (DNA) has also been used for measuring the living biomass in the sea

[1]Bedford Institute Contribution No. 207.

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(Holm-Hansen et al., 1968); however, this method has been criticized by Holm-Hansen (1969) in that its occurrence in oceanic samples was much too great to account for living particulate material properly.

Two cruises of CSS *Hudson* in 1968 from about 44°N to 19°N provided an opportunity to make observations on living and nonliving fractions of particulate matter in the open waters of the northwest Atlantic using chlorophyll, ATP, DNA, particulate organic carbon, C^{14}, and Coulter Counter methods. The present paper incorporates interrelations between the variables in addition to our findings on latitudinal variations in productivity and standing stock of algal material.

MATERIAL AND METHODS

Seawater samples from the surface were collected from January 25 to February 5, and from October 28 to November 7, 1968, at stations shown in Fig. 1. Sampling was done mostly with a clean plastic bucket from near the bow while the ship was underway, thus avoiding contamination

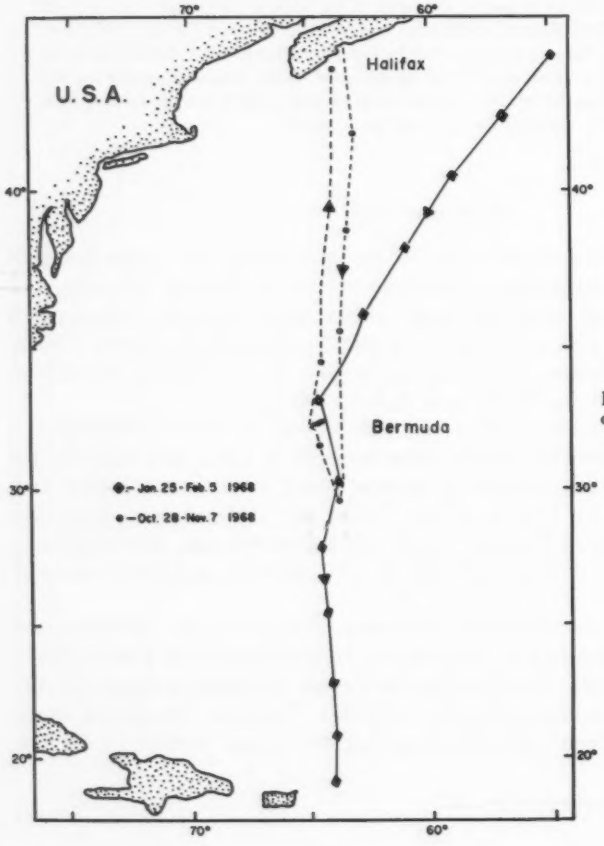

FIG. 1. Winter and fall cruise tracks and stations of CSS *Hudson*.

from the ship. On the winter cruise, some subsurface samples were also collected with a Niskin bottle. All the water was screened through 160-μ netting to remove large zooplankton. Samples for ATP concentration were filtered through 0.45-μ Millipore filters, frozen, and later analysed by the method of Holm-Hansen and Booth (1966). Samples for DNA were filtered, frozen, and subsequently analysed according to the method suggested by Holm-Hansen et al. (1968). During the winter cruise, samples for particulate organic carbon determination were filtered through 0.8-μ silver filters, vacuum-dried, and analysed according to the procedure outlined by Wangersky and Gordon (1965). For samples from the fall cruise, a Hewlett Packard carbon analyzer (Model 185, F & M Scientific, Avondale, Pennsylvania) was used. Samples for chlorophyll *a* estimation were filtered through glass fiber filters (Whatman GF/C), treated with magnesium carbonate, and kept frozen until analysis by a fluorescence method (see Strickland and Parsons, 1968).

Particulate volume determinations were made with a model B Coulter Counter equipped with a model M volume converter over the range 0.79- to 40.3-μ equivalent diameter, using apertures from 30 to 280 μ as required (Sheldon and Parsons, 1967). Particles larger than 40.3 μ could not be counted accurately because there were too few in suspension. However, the counts included most of the living particulate material less than 160 μ as evidenced by 79% of ATP and 84% of chlorophyll passing through a 10-μ nylon mesh. For growth experiments, incubation in 4-liter pyrex bottles was carried out on the open deck in a tub of running sea water for 24 hr, and particle concentration determined with a Coulter Counter before and after the incubation. Concurrent productivity experiments using carbon-14 (Steeman Nielsen, 1952) were carried out in 120-ml pyrex bottles.

RESULTS AND DISCUSSION

Values of the measured variables for the two cruises are plotted in Fig. 2. As expected, all values except temperature generally decreased from north to south — from eutrophic northern or nearshore waters to oligotrophic offshore southern waters. The interrelations of some of the variables measured are of interest.

The relation between carbon as estimated from chlorophyll concentration ($\times$100, Holm-Hansen, 1969) and carbon as estimated from ATP concentration ($\times$250, Holm-Hansen, 1969) is shown in Fig. 3B. The ratios between the two estimates varied from 0.3:1 to 1.2:1, a fourfold difference (Fig. 3A), and were relatively greater in the northern temperate waters. Holm-Hansen (1969) indicated that this will vary with species composition and environmental conditions. However, it may also reflect a relative increase of heterotrophic organisms (and hence a relative decrease of chlorophyll) towards the tropics (Kriss et al., 1964).

The amount of carbon representing living material (as calculated from ATP concentration) relative to total particulate organic carbon is shown in Fig. 4B. A difference in the ratio of total to living carbon between fall and winter samples can be seen (Fig. 4B), and in both sets of data the ratios range from 1.1:1 to 7.1:1, with a mean of 2.4:1; however, there seems to be no obvious latitudinal variation (Fig. 4A). These ratios are of about the same order and are in agreement with surface values at two Pacific stations given by Holm-Hansen (1969).

Holm-Hansen et al. (1968) have suggested that DNA concentration may be used as a possible measure of biomass. We found good correlation between ATP concentration and DNA concentration for both the cruises

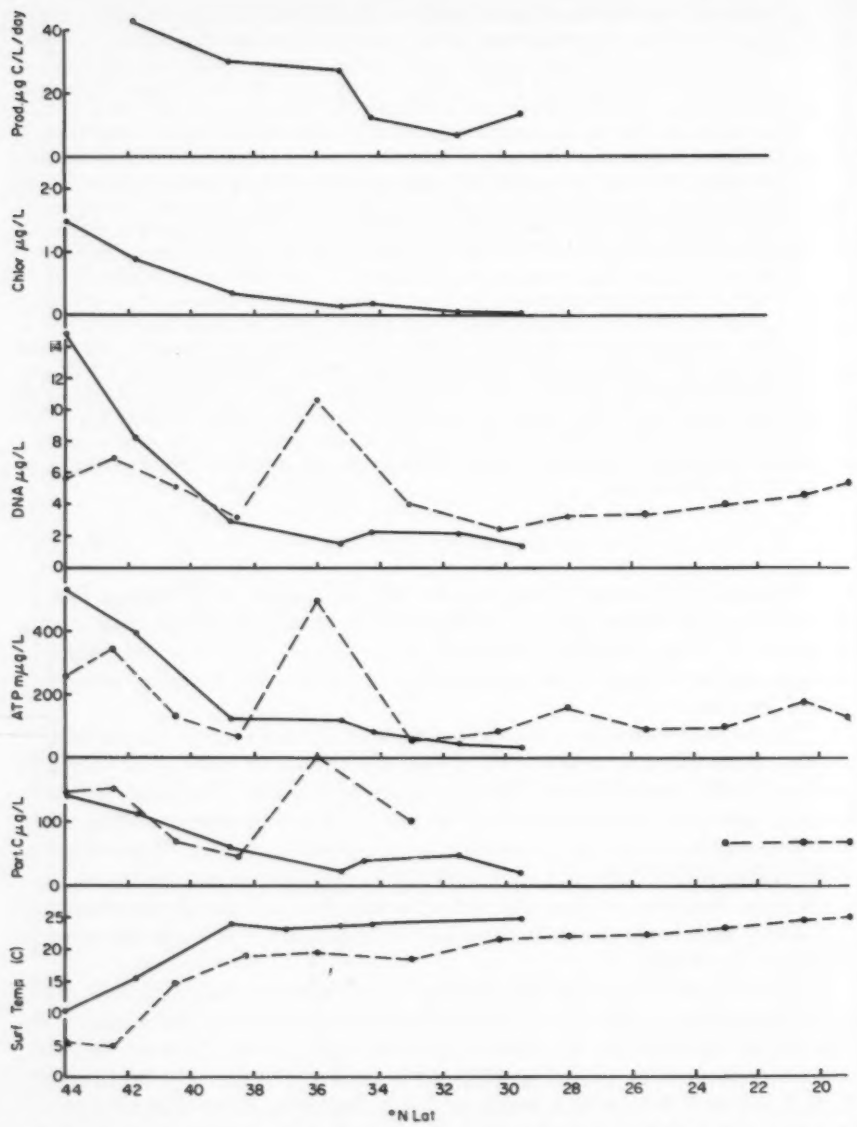

FIG. 2. Plots of measured variables with latitude; cruise of January broken lines, cruise of October solid lines.

(Fig. 5). However, in spite of this good correlation, DNA by itself appears to be of limited use as a measure of biomass or living carbon in oceanic populations. One of the main arguments against the DNA method is that the amount

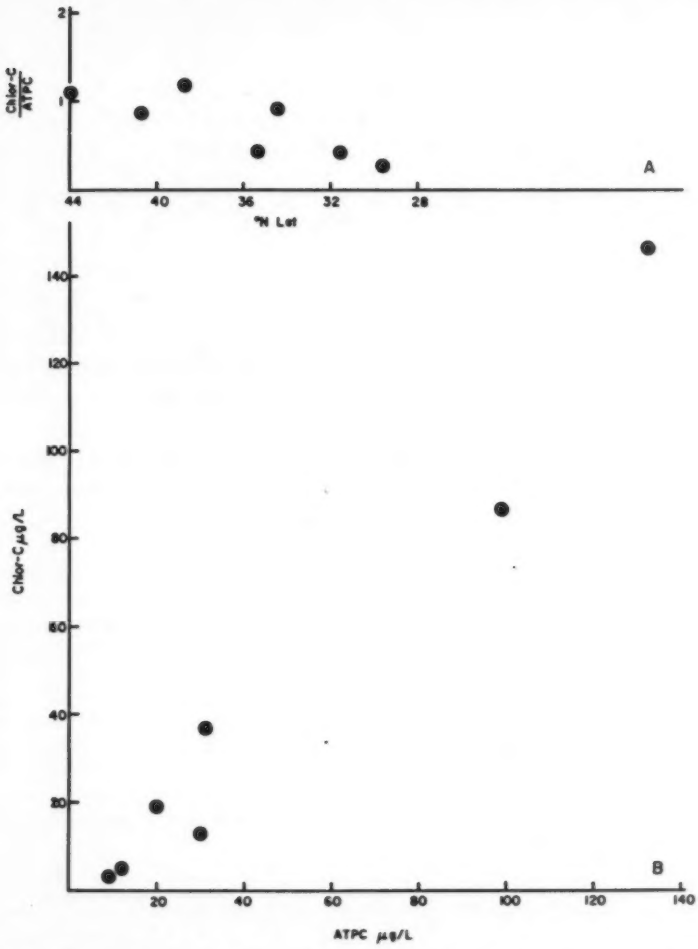

FIG. 3. (A) Chlorophyll carbon/ATP carbon ratios as a function of latitude, October 1968; (B) relation between chlorophyll carbon and ATP carbon, October 1968.

of living particulate carbon calculated from the DNA concentration (×50, Holm-Hansen, 1969) is greater than the amount of total particulate carbon. It would seem that DNA is associated with nonliving particulate material in natural waters.

Results of incubation experiments carried out during the October cruise are presented in Table 1. Here ATP is used as a measure of biomass (A) and the increase in particle volume as a measure of growth (B). The conversion of particle volume to carbon was achieved using the formula of Mullin et al.

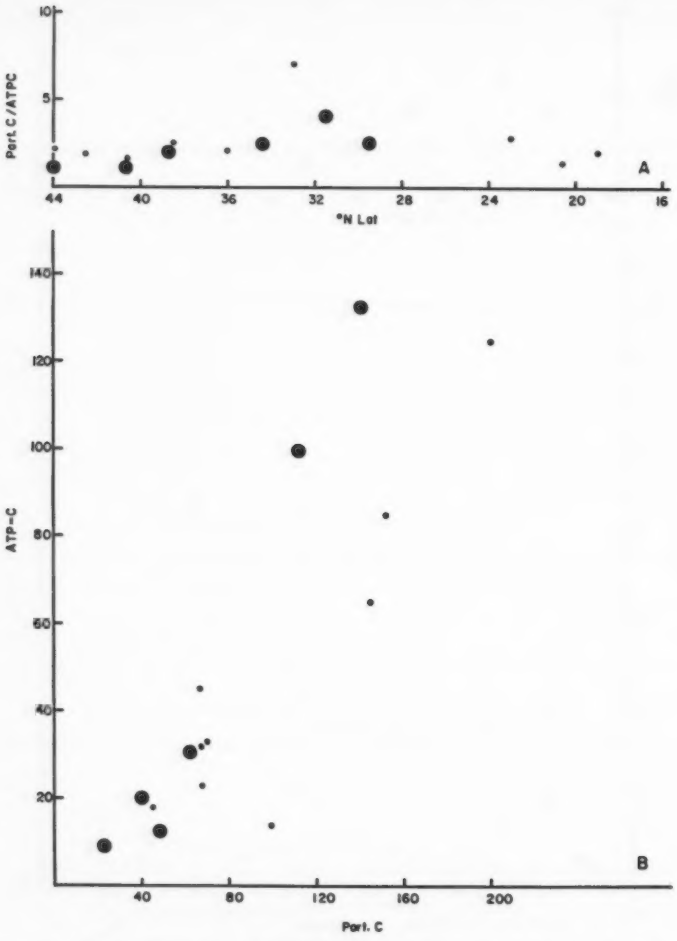

FIG. 4. (A) Particulate organic carbon/ATP or biomass carbon with latitude; (B) ATP carbon vs. total particulate organic carbon (μg/liter). January, small dots; October, large dots.

(1966). Ideally, of course, an individual conversion factor should be calculated for every size class, but since most of the growth centered around 8-μ diam, a standard conversion factor of 0.13 was used throughout. This is also very close to the factor (0.1) estimated by Zobell (1963) for converting marine bacteria (average volume 0.2 μ^3) volume to carbon.

Production estimates obtained by the Coulter Counter (Table 1B) were higher than those obtained by the C^{14} method and, in one instance, over seven times higher (Table 1D). There is no reason to suspect that the C^{14} values

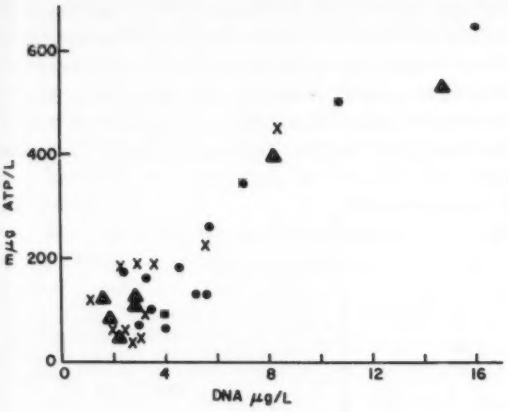

FIG. 5. Relation between ATP and DNA concentrations during the two cruises: January surface, ●; 25 m, ×; October surface, ▲.

TABLE 1. Results of 24-hr deck incubation experiments, cruise October–November 1968.

Location	A Initial biomass from ATP (μg C/liter)	B Growth from counter (μg C/liter/day)	C Specific growth rate (μg C/μg C/liter/day)	D Growth (μg C/liter/day C^{14})
43°59′N, 64°39′W	133	–	.20[a]	31.7
41 40, 64 02	99	51	.52	27.1
38 42, 63 50	31	34	1.1	5.4
35 18, 63 58	30	31	1.0	4.0
34 14, 64 29	20	14	.70	6.0
31 39, 64 30	12	7.3	.61	3.8
29 22, 64 03	9	15	1.7	2.7

[a]This value from ATP increase.

were in error. They were uncorrected for dark uptake, but those in the Sargasso Sea agree fairly well with other determinations at the same time of year (0.8–5.0 μg C/liter per day).[2]

The difference perhaps lies in nonphotosynthetic growth included by the Coutler Counter but not by the C^{14} method. There is the additional possibility that the particle volume measurements were complicated by fluxes of nonliving material (see Batoosingh et al., 1969); however, there seems to be no way of dealing with this problem with the information at hand.

Also presented in Table 1C are estimates of specific growth rate $\frac{\text{increase in carbon/liter per day}}{\text{biomass carbon/liter}}$ based on growth by Coulter Counter and

[2]Data of D. Menzel, "Panulirus" station (32°10′N, 64°30′W), on file at National Oceanographic Data Center.

standing stock by ATP. Specific growth rate is inversely related to biomass or standing stock. Although the data are few, they make interesting comparison with oceanic material from other sources. Figure 6 shows the growth rate vs. standing stock from data of Riley et al. (1949, table IV, $r = 0.72$, $P < 0.01$) and Fig. 7 shows our October data from Table 1A and C ($r = 0.80$, $P < 0.03$), together with material from the Gulf of Panama (Smayda, 1965), Sargasso

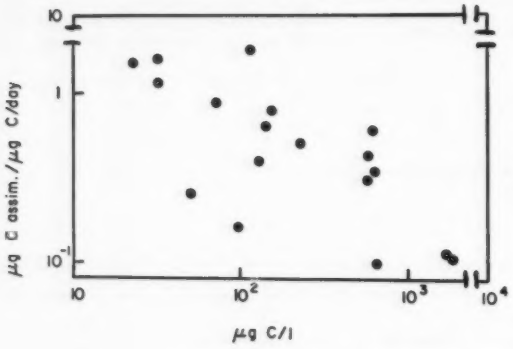

Fig. 6. Phytoplankton productivity vs. standing crop, various Atlantic localities (means of 216 points) (Riley, 1949, table VI).

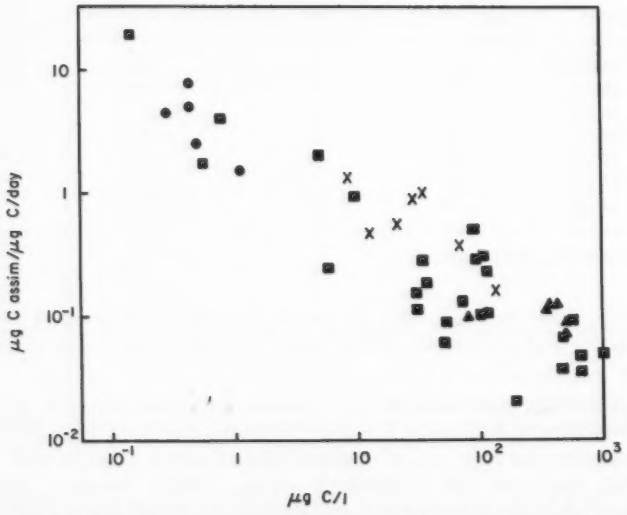

FIG. 7. Phytoplankton productivity vs. standing crop for: ■ Gulf of Panama (Smayda, 1965); ▲ Long Island Sound (G. A. Riley, personal communication); × October *Hudson* cruise; ● offshore Bermuda (Hulburt et al., 1960) using measurements from Smayda. Where species were not measured, the following volumes (μ^3) were used: unidentified pennate diatoms — 1207; unidentified centric diatoms — 22,366; *Chaetoceros danicus* — 6693; *Eucampia* sp. — 6000; *Lauderia borealis* — 21,000; *Stigmophora rostrata* — 1963; *Thalassiosira rotula* — 620; coccoliths — 130 (as *C. huxleyi*) (all preceding from Smayda, personal communication); dinoflagellates — 10,000 (G. A. Riley, personal communication).

Sea (Hulburt et al., 1960), and Long Island Sound (G. A. Riley, personal communication). There is a broad species overlap between Bermuda (Sargasso Sea) and the Gulf of Panama. Hulburt's surface counts were converted to volume with Smayda's measurements and then to carbon according to Smayda; this measure of biomass was then used with C^{14} uptake where information was available.[3] The three independent methods for measuring productivity and biomass all show this inverse relation between standing stock and productivity.

Each method has some difficulties and objections. The material of Riley et al. (1949) uses oxygen production and plant pigments for standing stocks. Smayda used C^{14} and microscopic measurements with counts for standing stock (also applied to the data of Hulburt et al.). However, they used one conversion factor to carbon for a wide range of cell sizes and carbon varies with cell size, as previously noted (Mullin et al., 1966). Our method uses the Coulter Counter for growth and ATP for standing stock; the counter may include changes in nonliving material.

Although the results are not readily converted to the units we used, an inverse correlation between productivity and standing stock is evident in data for fresh waters (Verduin, 1959; Wright, 1960; Findenegg, 1966).

The inverse relation of productivity to standing stock is not easy to relate to environmental factors in our data or Smayda's (1965). Important, perhaps, is cell size wherein the smaller and faster-growing cells (Williams, 1964; Eppley and Sloan, 1966) become more important in southerly waters (Saijo and Takesue, 1965; Pomeroy and Johannes, 1968).

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Taste Responses in Atlantic Salmon (*Salmo salar*) Parr

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SUTTERLIN, A. M., AND N. SUTTERLIN. 1970. Taste responses in Atlantic salmon (*Salmo salar*) parr. J. Fish. Res. Bd. Canada 27: 1927–1942.

External receptors located in the snout region of Atlantic salmon parr are innervated by the facial nerve and are differentially sensitive to several chloride salts ($NaCl = KCl > MgCl_2 > CaCl_2$). They are also sensitive to mineral and organic acids but insensitive to uncharged molecules such as neutral amino acids and simple sugars.

The palatine organ containing taste buds situated on the roof of the mouth is sensitive to strongly ionized as well as uncharged molecules.

The two classes of receptors differ in temporal patterns of discharge and in their susceptibility to the blocking agents Hg^{++} and Pb^{++} and the potentiating agent Cu^{++}.

Salmon parr can detect Hg^{++} in concentrations lower than other divalent cations as judged electrophysiologically; food pellets treated with dilute solutions of Hg^{++} are rejected by the fish.

As chain length increases in the aliphatic acid series the compounds become increasingly stimulatory as measured by nerve discharges in the palatine nerve.

Inert carriers treated with aliphatic acids of increasing chain length become increasingly preferred by salmon, and carriers treated with valeric and caproic acid are ingested.

Anosmic fish are capable of discriminating chemically treated carriers in a way similar to fish in which the olfactory organ is intact.

The cellular nature of external receptors of the snout is uncertain; the possibility that they are neuromasts is discussed.

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INTRODUCTION

MOST BEHAVIOURAL and electrophysiological studies of chemoreception in salmonids have dealt mainly with olfaction in relation to its role in migration and homing; gustation has to a large extent been ignored.

This investigation was undertaken to determine the cranial nerves and sensory structures involved in taste, to screen a variety of compounds by electrophysiological methods in an attempt to find out what salmon can taste, and to elucidate the possible role of gustation in the feeding behaviour of young salmon.

MATERIALS AND METHODS

Atlantic salmon, *Salmo salar*, were obtained as parr from a local hatchery where they had been hatched from native breeding stock taken from the Northwest Miramichi River, N.B. They were held in large tanks under natural daylength for approximately 4 months during which time they were maintained on beef liver and commercial fish food. At the time of the experiments they had reached a length of 12–15 cm.

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Electrophysiology

The branch of the facial nerve innervating the snout region on the upper jaw was exposed by removing the eyeball. A previous injection of tubocurarine had immobilized the animal and the snout was forced through an orifice in a rubber membrane to prevent flooding of the orbit during application of taste substances to the snout. The branch of the facial nerve containing fibers innervating chemoreceptors runs parallel with a larger branch of the trigeminal along the lower region of the orbit, and glass hooks were used to separate the two nerves from surrounding connective tissue. The smaller of the two nerves was placed on a bipolar silver–silver chloride hook electrode and the orbit was flooded with mineral oil to prevent the nerve from drying. The fish was perfused through the mouth with tap water during experimentation.

The method used to prepare the palatine organ for experimentation was similar to that described by Konishi and Niwa (1964). The palatine nerve, a branch of the glossopharyngeal, was exposed by decapitating the fish and removing the lower jaw. The upper jaw was then pinned to a paraffin block, and a portion of the skin on the roof of the mouth was stripped forward to expose the palatine nerve. This flap of skin was fixed to a glass rod with surgical glue (histoacryl) so that it formed a partition that prevented flooding of the electrode and nerve on the posterior side of the flap when taste substances were applied to the roof of the mouth on the anterior side of the flap.

Taste substances to be tested were drawn into disposable Pasteur pipettes that could be easily removed, filled, and inserted into a holder that held the pipette above the chemosensitive area. By means of a stopcock, air was admitted into the rubber tube attached to the pipette, and the test substances were allowed to flow over the chemosensitive area. A second tubing delivered distilled water continually as a rinsing medium. This flow was interrupted only during administration of the test substances.

Nerve discharges were amplified by a conventional, capacitatively coupled preamplifier and then led into a Grass, Model 7P3A, amplitude–frequency integrator (integrating time constant, 0.2 sec). This integrator was in turn coupled to a Grass, Model 7P10A, d-c integrator in order to measure the accumulated activity throughout the discharge. Both integrations were displayed on a Grass, multichannel, curvilinear pen-writing chart recorder. In some instances, photographs of the discharge were taken simultaneously with the above recordings on moving photographic paper using a Grass, Model C4, kymograph camera.

Because of the differences in responsiveness from one preparation to the next, a standard test substance was chosen to which the stimulatory effectiveness of other substances could be related. Concentrations of 0.1 M NaCl or $CaCl_2$ were most often used as both gave consistent responses without seemingly altering the sensitivity of the preparation to other substances. Changes in sensitivity of a preparation with time were taken into consideration by continual reference to the standard test solution.

Feeding Behaviour

Salmon parr held in captivity gradually learned to feed on commercial fish pellets. These pellets (3- to 4-mm diameter) floated on the surface for a period of time before becoming permeated with water. When a food pellet was added to a tank containing such conditioned parr, it was immediately struck, carried below the surface, and chewed until soft enough to crush and swallow. Depending on the initial hunger of the fish, a pellet might have been exchanged among several fish before it was finally consumed. Hungry fish rarely exchanged food pellets. However, as fish became progressively satiated, food pellets were exchanged more frequently before consumption. Inert, tasteless objects were similarly struck but seldom held longer than 1 or 2 sec.

Batches of 50 parr were placed in a 26-gal aquarium filled two-thirds full of water. Water flow in and out of the tank was approximately 1 gal/min. The tank was lighted from within and surrounded with black plastic except for a one-way window used for viewing.

Relative Preference for Treated Pellets

Commercial fish pellets were immersed for 15 sec in solutions of taste substances to be tested and then dropped into the aquarium. The number of exchanges of the pellet among different

fish before the pellet was finally consumed was noted, but the chemical nature of the test substances was coded and unknown to the observer.

The number of exchanges was taken as an index of the desirability of the taste substance in relation to the control. Because all pellets so treated are eventually consumed, presumably either distasteful substances gradually leach out, or the pellet eventually comes in contact with a "not so particular" fish.

Preference for Chemically Treated Inert Carriers

Small discs of polyurethane foam approximately the same size as commercial food pellets were completely saturated with taste substances and dropped into the aquarium. The carriers were immediately struck by the fish and the period of time that the carrier was held by the fish that struck it was used as an index of its taste attractiveness. The carrier in some instances was swallowed and in such cases the test substance was designated as highly attractive.

Because the above experiments on treated commercial pellets and treated carriers did not preclude the possibility that olfactory cues might have been involved, these behavioural experiments were repeated using salmon parr in which the olfactory organs had been bilaterally cauterized. Cauterization was done on anaesthetized fish viewed beneath a dissecting microscope. The olfactory rosette was cauterized, removed from the olfactory capsule, and the remaining stump of the rosette was charred flush with the pore of the capsule through which the olfactory nerve entered. Within 2 hr of the operation some of the fish were feeding, but a 24-hr period was allowed before resuming feeding experiments.

Histological Procedures

The heads of several salmon parr were fixed in Bouin's solution decalcified, sectioned serially at 10 μ, and stained with Mallory-Heidenhain solution. The external surface of the snout and roof of the mouth were specifically examined for taste buds and other receptors.

RESULTS

Facial Nerve–Snout Preparation

The method of obtaining data and the typical nature of the evoked discharge obtained from this preparation to the majority of active taste substances tested are shown in Fig. 1A. The background activity is immediately interrupted by a sustained discharge that is terminated by rinsing. Peak activity is reached in less than 500 msec; adaptation is relatively slow; and a rinse response, or distilled water effect, is never apparent. Application of salts exceeding 1.0 M concentration results in a second discharge (Fig. 1B). This response is slow in developing and often requires several minutes of washing to reduce the activity. This secondary response is most likely due to direct stimulation of the nerve endings innervating the receptors or stimulation of free nerve endings in the skin. Similar responses to high concentrations of NaCl could be obtained in branches of the trigeminal nerve innervating the skin of the head and containing tactile, but not chemosensitive fibers. The high concentrations of taste substances needed to cause this response were not generally employed in these experiments.

CHLORIDE SALTS

The magnitude of the responses evoked by a 1000-fold change in NaCl concentration is shown in Fig. 2. The level of peak activity increases with concentration in the same way that the level of total accumulated activity

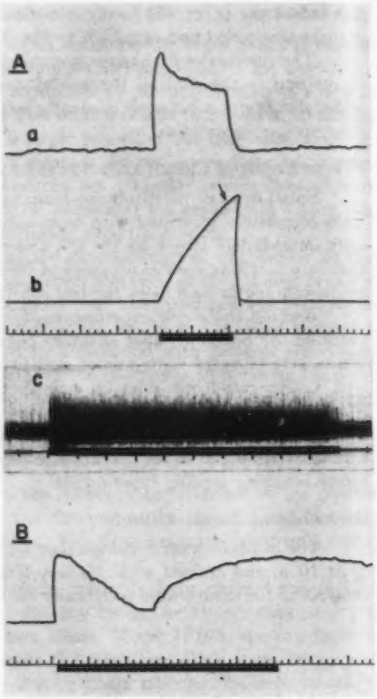

FIG. 1. (A) Typical response obtained from the facial nerve preparation showing methods of treating data: (*a*) output from amplitude–frequency integrator of the discharge shown in *c*; (*b*) simultaneous d-c integration of the above waveform. Arrow indicates level of activity accumulated in 10 sec of stimulation. The period of stimulation and time trace indicating 1-sec intervals is shown below; (*c*) discharge recorded from the branch of the facial nerve. The period of stimulation with 0.1 M NaCl and time intervals of 1 sec are indicated. (B) Amplitude–frequency integration showing early and late discharge evoked by 1.5 M NaCl; 1-sec intervals.

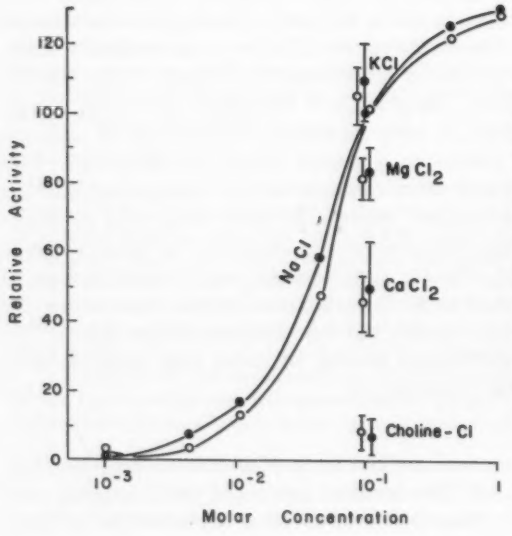

FIG. 2. Stimulation by chloride salts–facial nerve preparation. The concentration–response curve for NaCl was obtained from seven different preparations and all points are normalized as a percentage of that preparation's response to 0.1 M NaCl. The stimulatory effectiveness of 0.1 M KCl, $MgCl_2$, $CaCl_2$, and choline chloride was obtained from not fewer than 15 preparations and normalized with respect to that preparation's response to 0.1 M NaCl. Vertical rules and solid dots equal mean relative magnitude at peak activity ±2 SE; vertical rules and open dots equal mean relative magnitude of 10 sec of accumulated activity ±2 SE.

does during 10 sec of stimulation. In other words, $A = ch$ where A is the total accumulated activity, h is the level of peak activity, and c is an integration constant. This relation exists because both the rise time of the discharge and the rate of adaptation of the discharge remain constant at different concentrations of NaCl. Chloride salts of the divalent cations, Mg^{++} and Ca^{++}, are not so stimulatory as those of K^+ and Na^+; the large organic cation derived from the dissociation of choline chloride is the least stimulatory (Fig. 2).

ACIDS

The effectiveness as stimulants of various weak and strong acids at the same concentration is shown in Fig. 3. Comparison of integration methods

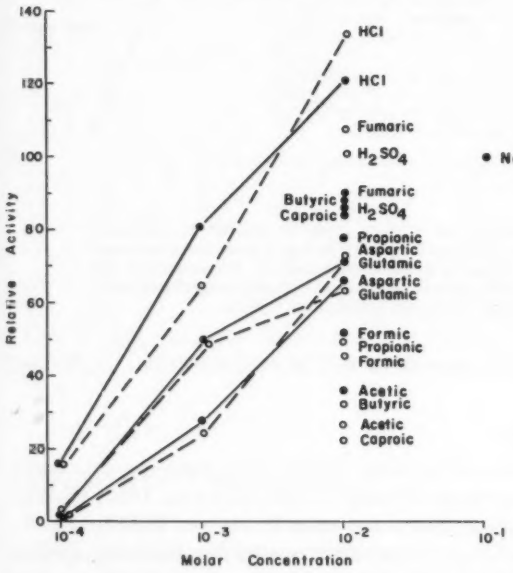

FIG. 3. Stimulation by acids—facial nerve preparation: solid dots are mean values of peak activity obtained from not less than 14 preparations with prior normalization as a percentage of each preparation's peak response to 0.1 M NaCl. Compounds closer than 10 relative activity units to each other do not evoke significantly different responses; open dots are mean values of 10 sec of accumulated activity obtained from not less than 14 preparations with prior normalization with respect to each preparation's accumulated response to 10 sec of 0.1 M NaCl. Compounds closer than 10 relative activity units to each other do not evoke significantly different responses.

shows that the "effectiveness" is different depending on the method of measuring the discharge. The differences are greatest in the homologous series of aliphatic acids. The response to the longer chain acids, although brisk, is only transient. The remaining compounds show similar ranking in both types of treatment.

Stimulatory effectiveness of the different acids is not specifically related to *p*H (replotted data, Fig. 4). Dissolved CO_2, caproic acid, fumaric acid, and sulfuric acid are equally stimulatory at widely different *p*H's.

NONSTIMULATORY COMPOUNDS TESTED

The following compounds were tested and evoked no detectable response: amino acids at 0.01 M concentration: DL-alanine, L-proline, L-histidine, L-hydroxyproline, L-isoleucine, DL-leucine, glycine, DL-methionine, DL-phenylalanine, DL-serine, L-threonine, DL-tryptophan, DL-valine, DL-taurine, and 10^{-3}

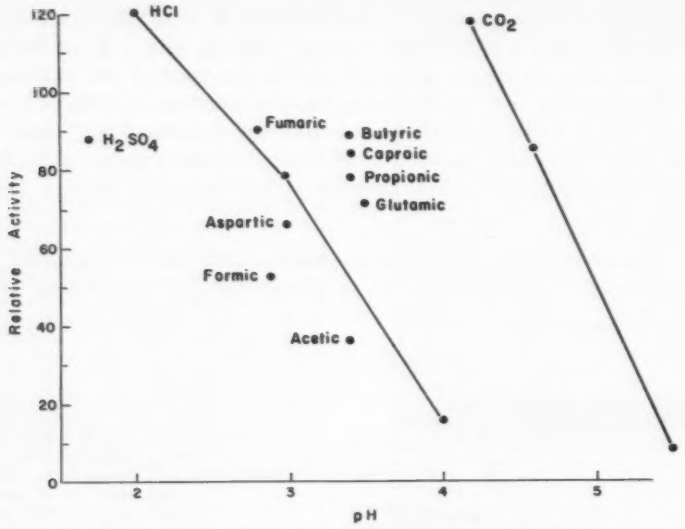

FIG. 4. Stimulatory effectiveness of various acids as related to *p*H-facial nerve preparation. Values for mean peak activity in Fig. 3 have been replotted against *p*H. All *p*H values shown are those obtained by measurement and do not vary more than ±0.2 *p*H units from those calculated using dissociation constants in the literature (Hodgman, 1962).

M L-tyrosine, and 10^{-4} M cystine; sugars at 0.1 M concentration: D-glucose, sucrose, lactose, maltose.

EFFECTS OF HEAVY METALS

The effects of heavy metals as blocking agents have been demonstrated in a number of chemosensitive systems (Deonier, 1938; Yur'eva, 1957; Hidaka and Yokota, 1967). The following ions were tested: Hg^{++}, Cu^{++}, Zn^{++}, and Pb^{++}. Only Hg^{++} and Pb^{++} were found to be effective blocking agents. Treatment for 10 sec with 0.001 M $HgCl_2$ followed by 1 min of distilled water rinse was usually sufficient to reduce by 50% or more the magnitude of both types of integrated responses evoked by a variety of taste substances (Fig. 5). There was no response by the preparation to $HgCl_2$ itself during the treatment. A similar depression of the taste response with more dilute solutions of $HgCl_2$ (0.0001 M) required periods of treatment longer than 10 sec. Treatment with 0.001 M $Pb(NO_3)_2$ was not so effective in blocking the response as was treatment with $HgCl_2$ (Fig. 5). Once the response was blocked by Hg^{++}, it remained so for long periods in spite of continued rinsing. However, a 10-sec treatment with 0.001 M $CuSO_4$, although evoking no response itself, completely removed the prior blocking effects of Hg^{++} or Pb^{++}, and the original response to the taste substance was renewed (Fig. 5). A potentiating effect of Cu^{++} was often seen in preparations that had not previously been treated with Hg^{++} or Pb^{++}, but the effect was not nearly so pronounced as those shown in Fig. 5.

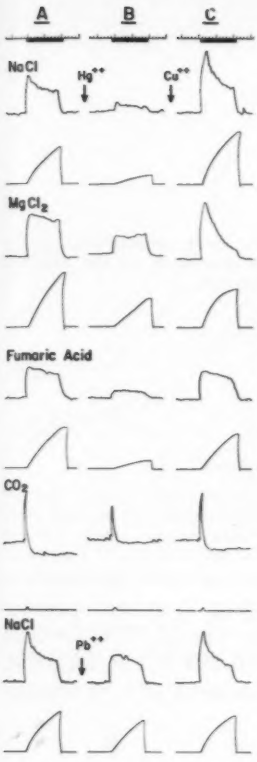

FIG. 5. Effects of heavy metals-facial nerve preparation: (A) initial taste response to a variety of substances; both types of integrated records are shown; time trace indicates 1-sec intervals; concentrations of test substances are NaCl 0.1 M, $MgCl_2$ 0.1 M, fumaric acid 0.01 M, CO_2 *p*H 4.6; (B) depression of taste response after 10-sec treatment with 0.001 M blocking agent (Hg^{++} or Pb^{++}) and 50 sec of distilled water rinsing; (C) restoration of response after 10-sec treatment with 0.001 M $CuSO_4$ and 50-sec distilled water rinsing.

PALATINE NERVE PREPARATION

The roof of the mouth of salmon parr in fresh preparations is very sensitive to both chemical and tactile disturbances and, because of the transient nature of the taste discharge, the two responses are rather difficult to separate. The tactile response, however, does not usually persist after the preparation has aged for a short time. The nature of the chemically evoked discharge differs in several ways from that obtained from the facial nerve preparation in that the discharge is very brief and a second discharge due to washing with distilled water is usually apparent. This distilled water effect occurs only if the receptors have previously been strongly stimulated, and varies in appearance from one preparation to the next (Fig. 6). Some preparations failed to exhibit any distilled water effect; in others the effect was usually present after stimulation with a variety of taste substances. Although this preparation was more stable with time than the facial nerve preparation, the discharge was more difficult to quantify because of its very brief nature. Therefore, only the amplitude of the integrated peak activity rather than accumulated activity was used as the index of stimulatory effectiveness.

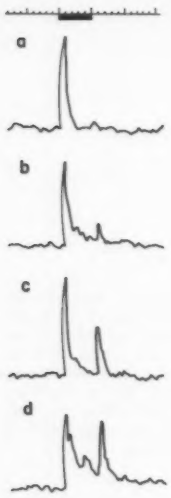

FIG. 6. Response of four different palatine nerve preparations (*a*, *b*, *c*, *d*) to 0.1 M $CaCl_2$ showing differences in magnitude of the distilled water effect; 1-sec intervals.

CHLORIDE SALTS

The ordering of the relative responses of the palatine nerve preparation to chloride salts ($CaCl_2 > MgCl_2 > NaCl = KCl$) (Fig. 7) is just opposite to that obtained from the facial nerve preparation (see Fig. 2). This reversal probably holds throughout the concentration range of 0.001–0.1 M, although only a few spot checks were made for the other ions besides Ca^{++} at concentrations lower than 0.1 M.

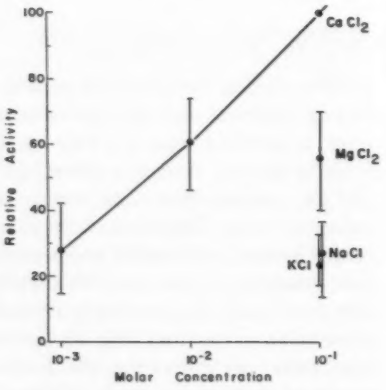

FIG. 7. Stimulation by chloride salts–palatine nerve preparation. The concentration response curves for the various salts were obtained from not less than 10 preparations and all points are mean values of peak activity with prior normalization as a percentage of each preparation's response to 0.1 M $CaCl_2$. Vertical rules and solid dots equal mean relative magnitude of peak activity ±2 SE.

ACIDS

The relative sensitivity of the palatine nerve preparation to a variety of weak and strong acids at the same concentration is shown in Fig. 8A. There is a striking increase in activity in response to aliphatic acids of increasing

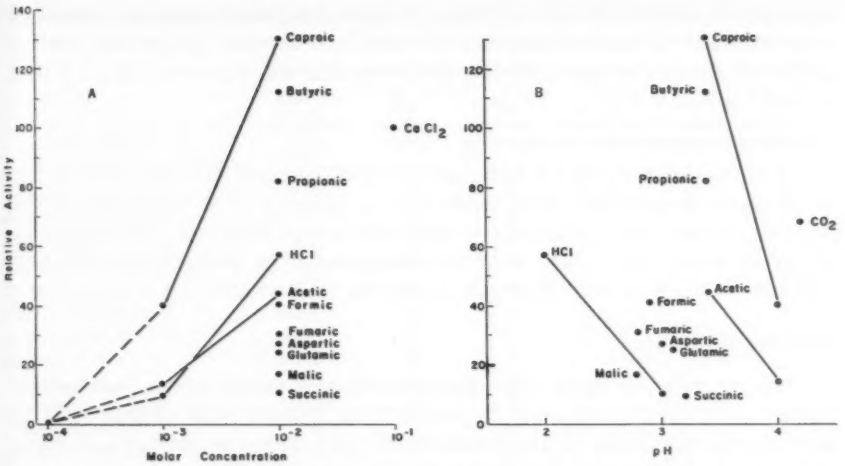

FIG. 8. Stimulation by acids–palatine nerve preparation: (A) relative amplitude of peak activity from not less than 14 preparations. Plotted points are mean values normalized as in Fig. 7. Compounds closer together than 15 units of relative activity do not evoke significantly different responses; (B) stimulatory effectiveness of various acids as related to *p*H. The *p*H values were obtained as described in Fig. 4.

chain length (Fig. 9). The strength of the various acids in terms of *p*H does not seem to account for their relative effectiveness as stimulants (Fig. 8B).

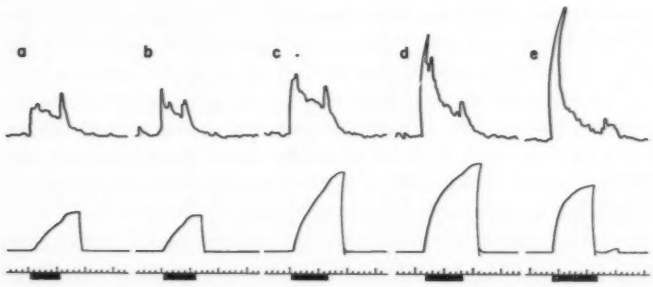

FIG. 9. Response to *n*-aliphatic acids of increasing chain length. Integration methods similar to Fig. 1; 1-sec intervals: (*a*) 0.001 M formic; (*b*) 0.01 M acetic; (*c*) 0.01 M propionic; (*d*) 0.01 M butyric; (*e*) 0.01 M caproic.

AMINO ACIDS AND CARBOHYDRATES

The 16 amino acids that failed to elicit responses in the facial nerve preparation were tested on the palatine nerve preparation at those concentrations used for the facial nerve–snout preparation, and only proline consistently produced a response. D-Glucose (0.1 M) was more stimulatory than sucrose; lactose and maltose produced no responses. Aqueous extracts of liver, commercial fish food pellets, and meal worms produced responses. From palatine

nerve preparations in three brook trout, *Salvelinus fontinalis*, however, responses were obtained to proline, alanine, tyrosine, and leucine. In general the response to all the compounds listed in this section was less than 50% of that evoked by 0.1 M $CaCl_2$.

EFFECTS OF HEAVY METALS

Treatments with Hg^{++} sufficient to block subsequent responses in the facial nerve preparation were ineffective in altering such responses of the palatine preparation to a variety of taste substances. However, concentrations of $HgCl_2$ as low as 0.0001 M evoked responses. The potentiating effect of 0.001 M $CuSO_4$ was seen, although it was not so pronounced.

Feeding Behaviour

Salmon parr in which the olfactory organ is intact reject food pellets treated with Hg^{++} and seem to prefer those treated with caproic acid to untreated pellets (Table 1). Inert carriers treated with the longer chain aliphatic

TABLE 1. Mean number of exchanges of treated and untreated food pellets.[a]

Test solution	Olfactory organ intact			Olfactory organ removed
	Group 1 (54 fish)	Group 2 (49 fish)	Group 3 (50 fish)	Group 2 (46 fish)
Distilled water control	2.1	2.4	1.1	2.5
Caproic acid (0.01 M)	1.4[b]	1.4[b]	0.0[b]	1.6[b]
$ZnSO_4$ (0.001 M)	2.9	1.9	1.1	
Hexanol (0.1 M)	2.1	1.9	1.6	
$CaCl_2$ (0.1 M)	2.1	2.4	1.6	
$HgCl_2$ (0.001 M)	3.3[b]	4.4[b]	3.3[b]	4.2[b]
$MgCl_2$ (0.1 M)	2.2	2.0	1.3	
$CuSO_4$ (0.001 M)	2.4	2.7	1.1	
Acetic acid (0.01 M)	1.9	2.0	1.4	
$Pb(NO_3)_2$ (0.001 M)	3.1[b]	2.4	1.8	

[a]In group 1, 24 pellets were treated with each compound; in group 2 with olfactory organ intact, 18 pellets; in group 3, 7 pellets; in group 2 with olfactory organ removed, 20 pellets.
[b]Difference significant, $P < 0.05$ (t-test), between pellets treated with chemicals and those treated with distilled water.

acids (butyric, valeric, and caproic) are held by the fish longer than untreated carriers and some are ingested (Table 2). Carriers treated with various amino acids and sugars yield responses indistinguishable from those of the distilled water controls.

Salmon parr deprived of olfactory cues by cauterization of the olfactory organ react to treated food pellets and carriers in a way similar to that of intact fish (Tables 1 and 2).

TABLE 2. Reaction to polyurethane foam (carrier) treated with various solutions.

Test solution	Olfactory organ intact (56 fish)			Olfactory organ removed		
	Retention time[a]	No. carriers tested	No. carriers swallowed	Retention time[a]	No. carriers tested	No. carriers swallowed
Distilled water	1.5	30	0	1.8	30	0
Formic acid (0.01 M)	2.2	21	0			
Acetic acid (0.01 M)	2.2	20	0			
Propionic acid (0.01 M)	3.3[b]	18	0			
Butyric acid (0.01 M)	5.1[b]	23	0			
Valeric acid (0.01 M)	12.2[b]	24	4			
Caproic acid (0.01 M)	44.0[b]	30	23	33.0[b]	27	17
Sucrose (0.1 M)	1.5	21	0			
Maltose (0.1 M)	2.0	20	0			
Glutamic acid (0.01 M)	2.0	6	0			
Proline (0.01 M)	1.6	6	0			
Glycine (0.01 M)	1.5	6	0			
Serine (0.01 M)	2.6	6	0			

[a]Mean time (sec) that the carrier was held by the first fish that took it before rejecting it. Swallowed carriers not included in mean value.
[b]Difference significant ($P < 0.05$) from distilled water control by t-test.

DISCUSSION

NATURE OF CHEMORECEPTORS INNERVATED BY FACIAL NERVE

DeKock (1963) states that end buds are numerous along the gills and buccal cavity of Atlantic salmon but no mention is made of the presence or absence of external taste buds in this species. In addition to the rows of canal organs and large pit organs that are visible to the naked eye, histological examination of the snout of salmon parr during this investigation revealed a third type of end organ, which seems to be best described as the "naked" type of pit organ named by Herrick (1901). Sâto (1955) has described similar organs on the head of young rainbow trout as consisting of sensory hair cells and supporting cells. However, we could not in our histological sections discern the diagnostic features of all neuromasts (Herrick, 1903): the presence of sensory hairs or a cupula. The structures were few in number (seldom more than six along the snout) and were considerably larger than the typical taste buds along the roof of the mouth.

In approximately half the preparations of the facial nerve, sustained discharge could be evoked by perfusing the snout with jets of water. This mechanical sensitivity, presumably from canal or pit organs, was immediately abolished after the initial administration of electrolytes to the snout. By touching various regions of the snout with very small droplets of salt solution from a fine glass capillary, the chemosensitive area was estimated to be the posterior portion of the snout containing canal and pit organs and not the extreme tip of the snout. Several attempts to isolate single fiber recordings

from the facial nerve in order to test for mechanical and chemical sensitivity failed. It should be mentioned that the posterior lateral line of salmon parr, which was examined briefly, was highly sensitive to water jets directed at the canal but insensitive to chloride salts.

Branches of the facial nerve in fish innervate a large portion of the acoustico-lateralis system and a number of investigators have shown that receptors of this system in certain teleosts and elasmobranchs are sensitive to ionic changes. Katsuki and Hashimoto (1969b) recorded responses from the lateral line of a number of freshwater and marine teleosts and found that the stimulatory effectiveness of chloride salts could be ranked in the following order: KCl > NaCl > LiCl. The salts of divalent cations ($CaCl_2$ and $MgCl_2$) were not stimulatory and inhibited the responses to the above salts when mixed with the test solutions. The receptors were demonstrated to be associated with pit and not canal organs. However, the discharges were much slower in developing (often requiring several seconds) than those found in the facial nerve preparation of salmon during this investigation.

Pit organs and ampullary organs of sharks and rays have also been shown to be sensitive to monovalent cations and to changes in saltwater concentration (Murray, 1962; Loewenstein and Ishiko, 1962; Katsuki and Hashimoto, 1969a). Although it cannot be said with certainty that the activity recorded in our studies was in fact from neuromasts, the concept of the acoustico-lateralis system acting as a behaviourally important chemoreceptor is open to question. According to Herrick (1903), fish in which both neuromasts and terminal buds are innervated by the facial nerve have, as the origin of the nerve fibers, entirely different parts of the brain and it is doubtful that the gustatory and acoustico-lateralis systems have much in common functionally, phylogenetically, or ontogenetically.

The possibility that free nerve endings are the receptive element cannot be overlooked. Bardach and Case (1965) showed behaviourally that the searobin could detect food using the free fin rays of its pectoral fins, which are entirely devoid of taste buds and contain only free nerve endings and innervated "spindle" cells in the epidermis (Bardach, 1967). Recordings from the spinal nerve innervating these fins yielded responses to NaCl, acetic acid, and several neutral amino acids but no response to sugars. Whether the free nerve endings or spindle cells are the true receptors remains to be demonstrated.

The physiological role of the various types of receptors as related to true gustation and general chemical sensitivity of the skin of fish is somewhat uncertain.

Comparative Chemical Sensitivity of Palatine Organ of Salmon and that of Other Fish

The multi-unit response-concentration curves for mineral salts that have been examined in a number of fish seem to be of two types. In the palatine organ of both carp and sea catfish maximum sensitivity is seen at low concentrations (0.001–0.002 M) of chloride salts of monovalent cations. At the most

dilute concentrations the magnitude of the response is highly dependent on the nature of the anions. At slightly higher concentrations activity is depressed, but at still higher concentrations a second peak in sensitivity is observed. This seems to be due to two different groups of receptors responding in different concentration ranges (Konishi and Niwa, 1964; Konishi, 1966; Konishi and Hidaka, 1967). However, concentration curves obtained from the bullhead barbel (Tateda, 1961) and the palatine organ of salmon parr in the present study more closely resemble those obtained in mammals (Pfaffmann, 1941; Beidler, 1953) in that the curves approach a maximum at relatively high concentrations (0.1–1.0 M) and the magnitude of the response is highly dependent on the nature of the cation.

The palatine organ of the carp (Konishi and Zotterman, 1963; Hidaka and Yokota, 1967), the pelvic fins of the tomcod, and the barbels of the bullhead (Bardach et al., 1967) respond to various common sugars and some neutral amino acids. The palatine organ of salmon was not especially sensitive to these classes of compounds.

The ionic species involved in stimulation by weak and strong acids is not completely understood in fish. Unlike the responses during stimulation with chloride salts, the cation concentration (H^+) does not alone determine the stimulatory effectiveness of the acids. Responses obtained from the palatine, facial, and branchial nerves of the sea catfish and carp, evoked by CO_2, HCl, and acetic acid (Konishi et al., 1969), were similar to those seen during this study in salmon. At the same *p*H, dissolved CO_2 was more effective than other acids, and by employing buffer systems and cross-adaptation Konishi et al. (1969) concluded that undissociated molecules of weak acids as well as the dissociated anion probably contribute to the response. Similarly, Tateda (1966) found differences in the stimulatory effectiveness of acids in the catfish barbel but postulated different groups of receptors specific for organic and mineral acids.

Effect of Heavy Metals

Hidaka and Yokota (1967) were able to depress subsequent responses to sugars from the palatine organ of the carp with 10-sec treatment with 0.0001 M $HgCl_2$. Because of the difference between the taste buds innervated by the palatine nerve and the obscure external receptors innervated by the facial nerve, Hg^{++} might have different effects on the two preparations in this investigation. The palatine nerve preparation showed high sensitivity to $HgCl_2$ (0.001–0.0001 M) compared to $CaCl_2$ and $MgCl_2$, and the subsequent responses from taste buds were not affected. On the contrary, the facial nerve preparation showed very little sensitivity to $HgCl_2$ at the same concentrations and the subsequent responses from the external receptors were blocked. The potentiating effect of Cu^{++} was seen in both systems. Mercuric ion is known to tie up sulfhydryl groups of proteins whereas cupric ion ties up amino groups. The respective depressing and potentiating effects of these metals on the

membranes or enzymatic components of the external receptors may be related to these properties (Baradi and Bourne, 1951; Landgren et al., 1953; Yur'eva, 1957). Since concentrations of $HgCl_2$ as low as 0.0001 M evoked responses in the palatine nerve and both normal and anosmic salmon parr showed a significant avoidance of food pellets and inert carriers treated with $HgCl_2$, possibly these fish can taste very dilute concentrations of Hg^{++}. However, no behavioural differences were noted when the fish were presented food pellets or inert carriers dipped in 0.001 M $CuSO_4$ despite the potentiating effect of this compound in both nerve preparations.

Role of Gustation in Feeding

The degree to which fish employ the various senses (visual, gustatory, and olfactory) in food detection and selection varies considerably among different species (Wunder, 1927, 1936). Salmon parr can probably be categorized along with some predacious fish as using visual cues for prey detection and gustatory cues as a final means of food selection after the prey has been seized.

The roof of the mouth of salmon parr is composed of dermal teeth between which are located folds of dermis containing taste buds. Our preliminary studies showed that the cuticle of ingested meal worms immediately recovered from the stomachs of salmon parr were always crushed or pierced in several places. To what extent the variety of invertebrates that compose the diet of young wild salmon are sampled in this way is uncertain. However, olfactory detection of the released juices from seized meal worms is not necessary for ingestion as anosmic salmon parr ingest meal worms also. Olfaction is also not necessary for discrimination of chemically treated inert carriers that have been seized.

None of the amino acids or sugars tested caused either increased retention or ingestion of polyurethane foam pellets, even though neural responses were obtained to some of these compounds. Bardach and Case (1965), however, found that perfusion of the fin rays and lips of anosmic searobins with several amino acids resulted in digging and swallowing movements, and search behaviour could be initiated in anosmic bullheads by releasing cysteine into the water (Bardach, 1967).

No apparent reason for the pronounced stimulatory action on ingestion of the longer chain aliphatic acids (valeric and caproic) is obvious to us, except that perhaps these compounds have properties approaching those of lipidlike substances, which as a class may be highly stimulatory. Konishi and Zotterman (1963) fractionated various components from a variety of natural compounds and the substances most active when tested on the palatine organ of the carp were phospholipids. Bardach et al. (1967) treated the barbel of the catfish with lecithin, cholesterol, and inositides, and neural responses were obtained to the latter two compounds.

Analysis of stomach contents of Atlantic salmon parr by Allen (1941) indicated that nymph, larval, and pupal stages of aquatic insects and, to a lesser extent, annelids compose the diet in early summer. This predominantly

bottom feeding behaviour changes in late summer to surface feeding on adult insects. Chemical extraction of the various classes of compounds from these insects and annelids and subsequent testing both neurophysiologically and behaviourally might provide a useful correlation between the gustatory activity of salmon parr in the laboratory and in the wild. Other factors governing food selection and intake such as the past feeding history in terms of food quantity and food quality (nutritive value, availability, as well as palatability) can be expected to be equally important.

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Parasitic Copepods of *Sebastodes alutus* Including *Chondracanthus triventricosus* sp.nov. and *Colobomatus kyphosus* sp.nov.

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SEKERAK, A. D. 1970. Parasitic copepods of *Sebastodes alutus* including *Chondracanthus triventricosus* sp.nov. and *Colobomatus kyphosus* sp.nov. J. Fish. Res. Bd. Canada 27: 1943–1960.

Sixty-six specimens of *Sebastodes alutus*, the Pacific ocean perch, collected from the coast of Oregon and British Columbia were examined for parasitic copepods. The six species identified are *Chondracanthus pinguis* Wilson, 1912, *Chondracanthus triventricosus* sp.nov., *Clavella parva* Wilson, 1912, *Brachiella robusta* (Wilson 1912) Kabata, 1970, *Colobomatus kyphosus* sp.nov., and *Sarcotaces arcticus* Collett 1874. *Colobomatus kyphosus* is the second species of the genus to be described from the eastern Pacific and *Ch. triventricosus* is the second chondracanthid to be found in nasal cavities. *Sarcotaces arcticus* is reported as a parasite of *S. alutus* for the first time. Details of the appendages of each new species are described and illustrated.

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INTRODUCTION

SIXTY-SIX SPECIMENS OF *Sebastodes alutus* Gilbert, the Pacific ocean perch, were collected during 1969 and examined for parasitic copepods. Of the six species recovered, two have not been previously described in the literature and another has not been reported from *S. alutus*. One of the new species, a chondracanthid, inhabits the nasal cavities; the other, found in the cephalic sensory canals of the head and in the canals of the dentaries, is referable to the genus *Colobomatus*.

The new species of copepods in the present work were described by Wilkes (1957) in a Masters thesis. Since his information has not been published, it seems reasonable to describe and report them at this time; particular attention has been given to details of the appendages that have been shown to be important in arthropod classification. Data on incidence and intensity of infection are also recorded for other species recovered in this study.

MATERIALS AND METHODS

Specimens of *S. alutus* were taken in May, June, and September 1969, off the coast of British Columbia from La Pérouse Bank near the southwestern coast of Vancouver Island and from Queen Charlotte Sound near the Scott Islands. Additional specimens were collected in September from the Oregon coast near Mack Arch. Sixty-six fish ranging from 27 to 44 cm fork length were examined fresh or were quick-frozen for later examination. Although complete detailed

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examinations were performed, it must be taken into account that the specimens were taken in a trawl at depths from 71 to 250 fath and some external mobile copepods might have been lost.

Live copepods were relaxed in magnesium sulfate before fixation and storage in 10% formaldehyde. Frozen specimens usually died in an extended position causing no great difficulty in later examinations. Dissection was normally necessary to obtain adequate views of the smaller appendages; these were mounted temporarily in glycerol, Berlese's fluid, lactic acid, or water. Various stains were used but none was found to be more useful than a simple clearing agent and proper illumination. All drawings were prepared with the aid of a drawing tube. Measurements are in millimeters unless otherwise stated. Ranges are indicated in parentheses after average measurements and intensities of infection. Incidences of infection are expressed as a percentage of the 66 specimens of *S. alutus* examined.

Chondracanthus ***triventricosus*** sp.nov.

(Fig. 1–24)

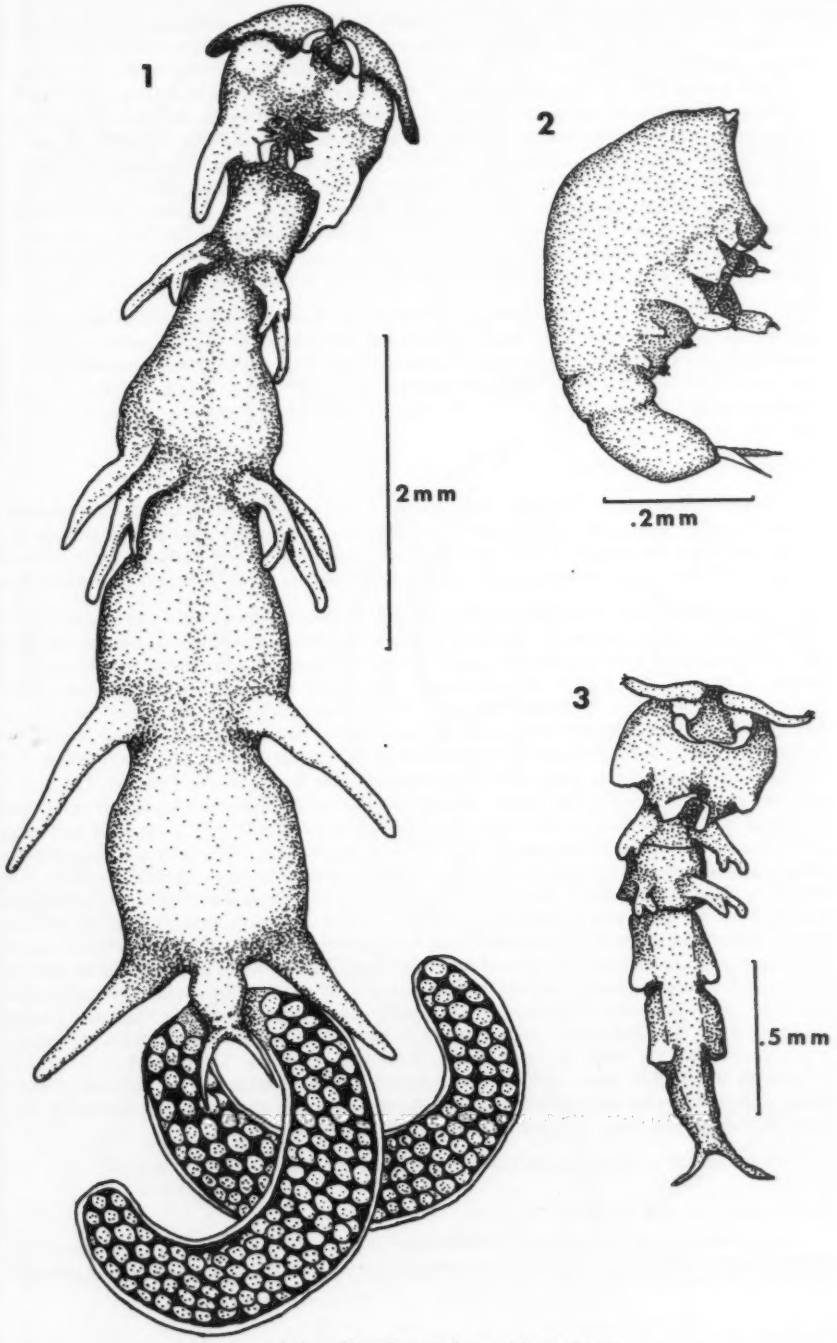
Deposition of specimens — One ovigerous female becomes the holotype, the attached male the allotype. Three additional ovigerous females also with attached males become paratypes. These specimens are deposited in the National Museums of Canada in Ottawa, Cat. No. NMC 15060.

Type host: *Sebastodes alutus* Gilbert. *Type habitat*: Nasal cavities. *Type locality*: La Pérouse Bank near the southwestern coast of Vancouver Island, B.C.

Description of the Female

General appearance — The general appearance of *Chondracanthus triventricosus* (Fig. 1) is slender and elongate with curled egg sacs. Although no distinct segmentation is evident, the body may be divided into cephalothorax, trunk, and genito-abdomen, with the trunk further subdivided by constrictions into three distinct swellings. The cephalothorax is slightly wider than long, and narrows posteriorly. The ventral surface is produced into small anterolateral swellings, and the posterolateral corners are extended to form cephalothoracic processes that extend posteriorly and terminate slightly anterior to the first pair of thoracic legs. These processes may be notched on the posterolateral margins as shown in Fig. 1. Each swelling of the trunk bears a pair of slender, conical lateral processes projecting from its posterolateral margins. The first two pairs of processes extend ventrolaterally whereas the posterior pair extend posterolaterally away from the body. The thoracic legs are biramous with the exopod longer than the endopod. The first pair of legs originate from a very slight swelling on the ventrolateral surface of the "neck"; the second pair extend from the posterolateral margins of the first conspicuous trunk swelling, medial and posterior to the first pair of trunk lateral processes. The genito-abdomen consists of a small outgrowth, one-fourth the width of the last trunk swelling, and a small rounded abdomen, visible dorsally, that bears the caudal furca. The egg sacs are attached to the posterolateral surface of the genital segment and may be coiled in various positions. They may describe a half circle or be more tightly coiled with their tips extending more anteriorly (Fig. 1). No distinguishing characteristics are present on the dorsal surface.

Appendages (*only pertinent parts illustrated*) — The first antenna is large, inflated, and devoid of segmentation. The distal portion narrows abruptly and curves posteriorly around the cephalothorax. Eight apical setae (Fig. 4) project from the tip; four subapical setae are located on the ventral surface. In addition there are two smaller setae (Fig. 5) on the ventral surface of the most anterior portion of the appendage. The second antenna, which serves as an attachment organ, arises from a conical protuberance and is modified to form a strong hook. The distal portion (Fig. 6) curves sharply, has a distinct indentation along its concave margin, and bears a longitudinal band of striations proximal to the notch. The mandible (Fig. 7) is armed with a dorsal and a ventral row of denticles; those of the dorsal row are finer and shorter. The first maxilla (Fig. 8) is armed with a stout terminal spine as well as a smaller subterminal spine on its medial



FIGURES 1–3. ***Chondracanthus triventricosus.***
Fig. 1. Female, ventral; Fig. 2. Male, lateral, first antenna not shown; Fig. 3. Juvenile female, ventral.

margin. The second maxilla (Fig. 9) has 12–19 denticles along its dorsal margin. Nineteen denticles are shown in Fig. 9 but more usual numbers are 12–14. In addition the maxilla bears a large smooth auxiliary spine and a small basal seta. The maxilliped is three-jointed and terminates in a strong claw (Fig. 10) that has two small denticles on the concave margin near its tip. It is also equipped with spiny pads that extend along the dorsal surface of the distal segment. The caudal furca (Fig. 11) bears one dorsal and two ventral setae near its base. There is also one seta on the lateral surface of the abdomen a short distance anterior to the bifurcation of the furcae. Each thoracic leg (Fig. 12) bears a single seta on its lateral margin near the bifurcation of the exopod and endopod. In addition the tip of the exopod (Fig. 13) is tipped with three sensory papillae. Short spines (Fig. 14) were observed on the lateral processes. Figure 24 indicates the position of other such spines; circles represent dorsal locations and dots represent ventral locations. These spines are very difficult to observe and Fig. 24 is a composite from many observations.

Measurements — The following measurements are based on 11 specimens with well-developed, attached egg sacs. Total length is measured from the most anterior portion of the first antennae to the bifurcation of the caudal furcae. Length of the cephalothorax excludes the first antennae. Total length 7.8 (6.3–9.9), cephalothorax 1.0 × 1.2 (0.8–1.2 × 1.0–1.4), trunk (including genito-abdomen) 6.4 × 1.5 (5.1–8.2 × 1.3–1.5), egg sacs 4.4 × .6 (3.1–5.2 × 0.4–0.8).

Description of the Male

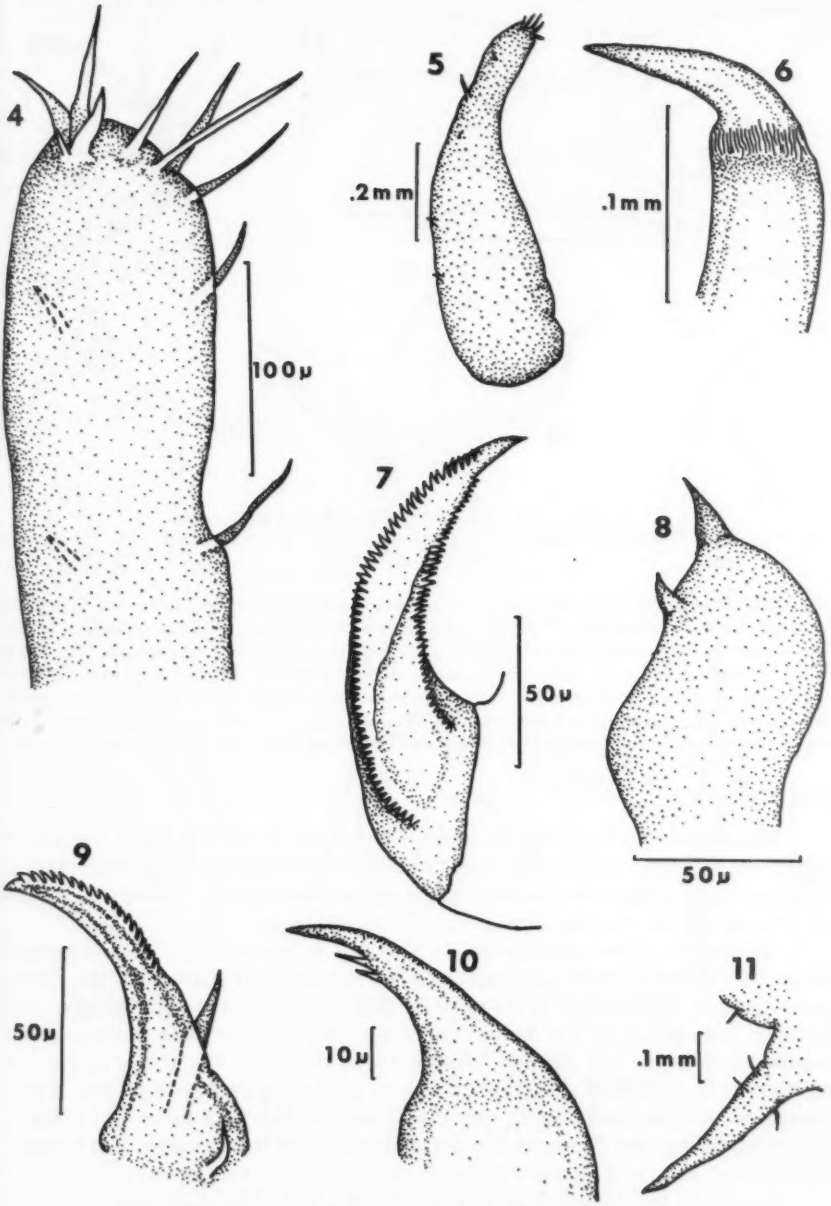
General appearance — The male of this species is very similar to other chondracanthid males. Viewed laterally (Fig. 2) the cephalothorax is brought to a prominent point from which the first and second antennae emerge. Posteriorly the trunk tapers and curves ventrally terminating in caudal furca. Faint signs of segmentation are present posterior to the last pair of thoracic legs.

Appendages — The first antenna arises slightly posterior to the second antenna and extends ventrolaterally, closely applied to the body. The tip (Fig. 15) is crowned with eight apical setae and one subapical seta on the anterior margin. Three additional setae are located on the anterior margin of the proximal half of the appendage. The second antenna (Fig. 16) originates from the most anterior portion of the cephalothorax and consists of a hamate "endopod" and an "exopod" that is tipped with two very short terminal spines. Although of substantial length, the "exopod" typically lies closely applied to the base of the endopod and is easily overlooked. The "endopod" is a very strong hook that lacks setae or other distinguishing features. The mouth parts are similar to those of the female although the following differences are evident upon close examination. The denticles on the dorsal margin of the mandible (Fig. 17) are stout and more widely spaced than those of the ventral row. The first maxilla (Fig. 18) carries a strong terminal spine, a smaller subterminal spine, and a small rounded protuberance on its medial margin. The second maxilla (Fig. 19) has a dorsal row of a maximum of 5 small denticles, a well-developed, smooth auxiliary spine, and a basal seta. Variation, probably due to wear, was found to occur in the number and shape of the denticles, some being merely rounded outgrowths whereas others were sharp and well defined. The claw of the maxilliped (Fig. 20) bears one well-formed denticle on its concave margin. The two pairs of thoracic legs (Fig. 21 and 22) that extend from the ventrolateral surface of the body are biramous, each bearing a long seta arising from the lateral margin of the protopod. The endopod of the first thoracic leg is short and rounded whereas the exopod is long and cylindrical. Each is armed with a short terminal spine. The exopod of the second thoracic leg is similar to that of the first but is tipped with three setae and the endopod narrows abruptly at its midpoint and terminates in an unarmed tip. As in the female, one dorsal and two ventral setae project from the base of the caudal furca (Fig. 23).

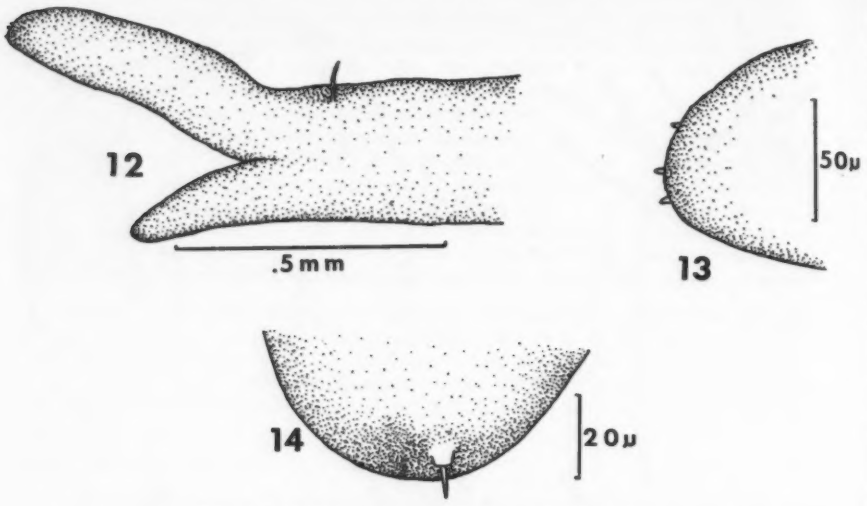
Measurements — Total length based upon eight specimens is 0.6 (0.5–0.6).

Description of the Juvenile Female

A juvenile female 1.5 in length is illustrated in Fig. 3. At this stage of development the cephalothorax is wider than long but is not tapered posteriorly. Cephalothoracic lateral processes



FIGURES 4–11. Female appendages, *C. triventricosus.*
Fig. 4. Tip first antenna, dorsal; Fig. 5. First antenna, ventral; Fig. 6. Tip second antenna; Fig. 7. Mandible, distal part; Fig. 8. First maxilla; Fig. 9. Maxillary claw, ventral; Fig. 10. Claw of maxilliped; Fig. 11. Caudal furca, ventral.



FIGURES 12–14. Female appendages, *C. triventricosus*.
FIG. 12. First thoracopod; Fig. 13. Tip of exopod of first thoracopod; Fig. 14. Tip of third lateral process.

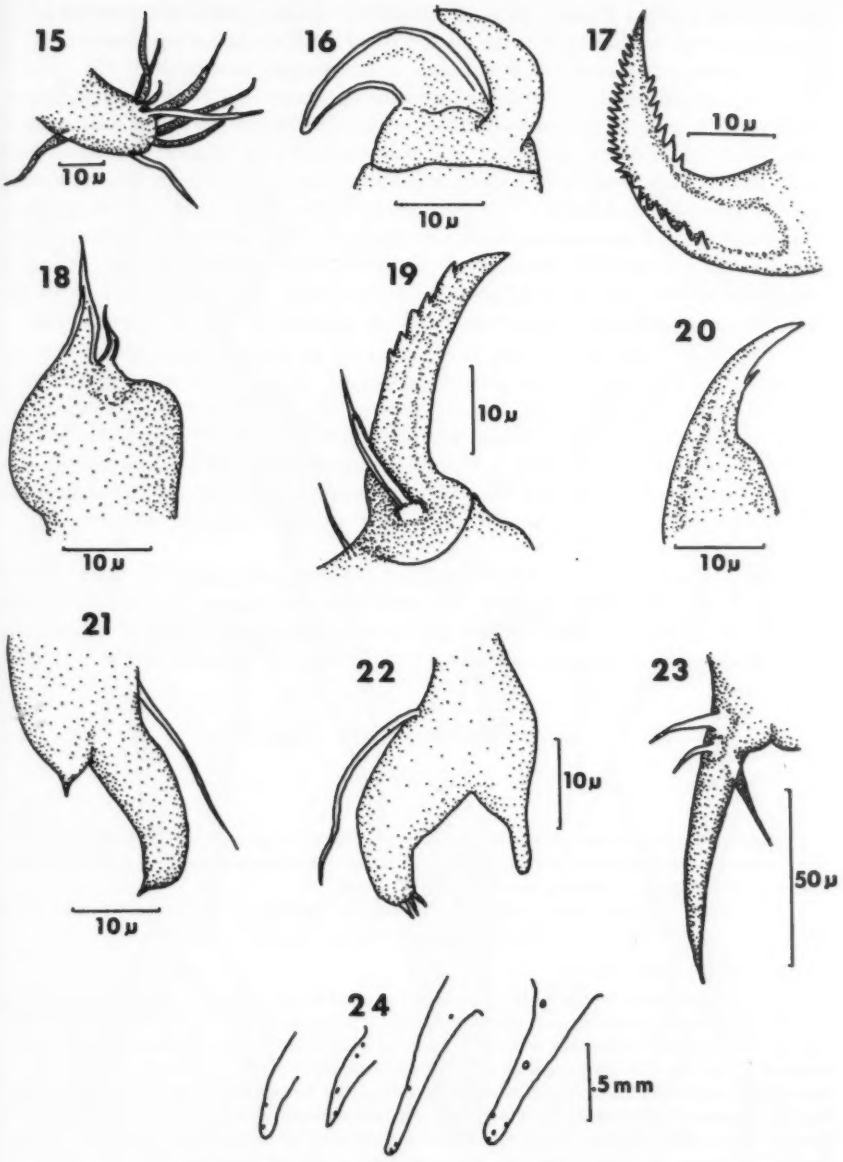
are indicated by small outgrowths on the posterolateral corners of the cephalothorax. Five trunk segments are well defined but the "neck" of the trunk is not yet developed. Small biramous thoracic legs extend from the posterolateral corners of the first two segments and the beginnings of the lateral trunk processes are indicated by slight protuberances on the posterolateral corners of segments 2, 3, and 4. The genito-abdomen is large in proportion to the juvenile size and is obscurely segmented. The appendages of the cephalothorax are large and well-developed although the first antenna is more slender than in the adult and the second antenna is not yet notched distally.

DISCUSSION

Kabata (1969) recently described *Ch. narium* from the nasal cavities of *Ophiodon elongatus* in British Columbia. Prior to this report no chondracanthid was known to utilize this habitat, hence *Ch. triventricosus* is the second species of the genus to be discovered from this location.

Distinctive characteristics of the female *Ch. triventricosus* are its general shape, the bulbous trunk swellings, and the number and position of the lateral processes, in addition to differences in the details of the appendages. The position and shape of the lateral processes and thoracic legs are somewhat similar to those of *Ch. pinguis* Wilson, 1912. However, the trunk of *Ch. triventricosus* is elongated and constricted to produce swellings and the lateral processes have no tendency to curve in a medial direction as in *Ch. pinguis*. The thoracic legs are also more slender and elongated than those of *Ch. pinguis*. Curved egg sacs is a characteristic shared only with *Ch. narium*.

Details of the appendages of the Chondracanthidae of British Columbia were studied by Kabata (1968). His work makes possible the following comparisons. The apical and subapical armatures of the first antennae resemble



FIGURES 15–23. Male appendages, *C. triventricosus.*

Fig. 15. Tip first antenna, ventral; Fig. 16. Second antenna, ventral; Fig. 17. Mandible, distal part; Fig. 18. First maxilla; Fig. 19. Maxillary claw, posterior; Fig. 20. Claw of maxilliped; Fig. 21. First thoracopod; Fig. 22. Second thoracopod; Fig. 23. Caudal furca, ventral.

FIG. 24. Spinulation of female lateral processes, *C. triventricosus.*

that of *Ch. gracilis* Fraser, 1920, although the author could not observe cones or evidence of any fusion of setae as described by Kabata for the above species. Kabata reported transverse striations on the second antennae of *Ch. gracilis* and *Ch. pusillus* Kabata, 1968 and smooth antennae in *Ch. irregularis* Fraser, 1920 and *Ch. pinguis*. The second antennae of *Ch. triventricosus* has longitudinal striations. Z. Kabata (personal communication) has observed longitudinal striations in other species of Chondracanthidae hence this characteristic should not be considered specific. The first maxilla resembles that of *Ch. gracilis*. *Chondracanthus triventricosus* and *Ch. pinguis* have similar maxillary claws in that the number of denticles is approximately equal and the auxiliary spine of each is smooth and tapers to a fine point. As in *Ch. irregularis* the claw of the maxilliped bears two denticles instead of the more usual one.

The male is similar in size and structure to others of the genus but especially to *Ch. pinguis*. The second antennae retain their exopods although no seta could be observed on the endopod of *Ch. triventricosus*. The maxillary claw of *Ch. pinguis* carries fewer denticles than that of *Ch. triventricosus* but is otherwise similar in having a smooth auxiliary spine and a basal seta. The claw of the maxilliped is like that of *Ch. pinguis* as are the thoracic legs with the exception of a more elongate exopod on the first thoracic leg.

Ch. triventricosus infected 81.8% of the fish examined. A total of 255 females were collected; of these 132 were juvenile stages. One hundred and thirteen males were found attached to the ventral surface of the genital segment of mature females. Average intensity of infection for females was 4.7 (1–14).

Colobomatus **kyphosus** sp.nov.

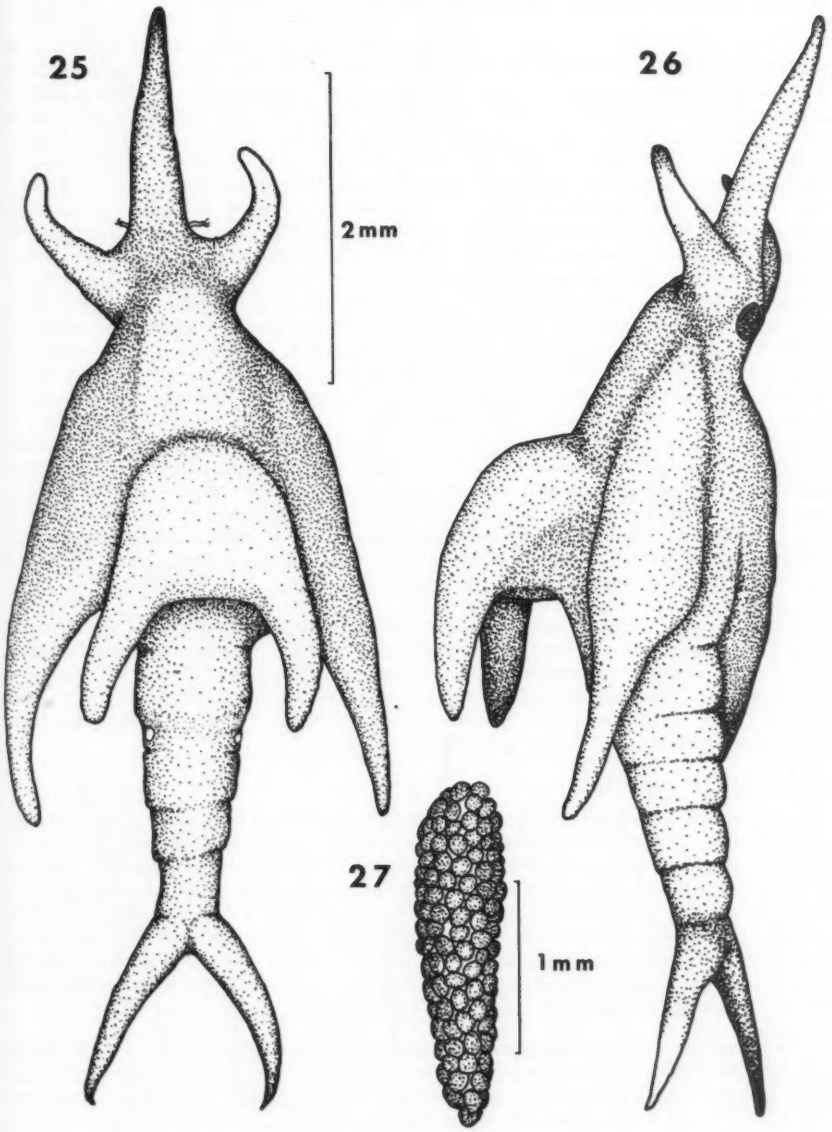
(Fig. 25–47)

Deposition of specimens — One female becomes the holotype and one male the allotype. Three females and three males become paratypes. These specimens are deposited in the National Museums of Canada in Ottawa, Cat. No. NMC 15061.

Type host: *Sebastodes alutus* Gilbert. *Type habitat*: Cephalic sensory canal system. *Type locality*: La Pérouse Bank near the southwestern coast of Vancouver Island, B.C.

Description of the Female

General appearance — The body (Fig. 25–26) is divided into cephalic, thoracic, and genito-abdominal portions by constrictions posterior to the buccal capsule and thoracic lobes. Anteriorly the cephalic portion is elongated to form a medial conical lobe. A pair of lateral cephalic lobes originate at the level of the buccal capsule, extend laterally, and then curve anterodorsally. A large dorsal outgrowth arises from the posterior half of the thorax and bifurcates, sending lobes from its posterolateral corners to extend over the anterior genital segments. A pair of lateral thoracic lobes originate from broad bases and extend winglike posteriorly, their tips curving dorsally. In dorsal view these lobes give the appearance of sloping shoulders. All lobes terminate in simple rounded tips. Five segments are clearly defined ventrally on the genito-abdomen, but the anterior segments are less well defined on the dorsal surface. Directly above the attachments of the egg sacs on the dorsolateral surface of the second segment are slight furrows that produce small enlargements directly above them, giving the impression of a true segment. The egg masses



FIGURES 25–27. ***Colobomatus kyphosus***, female.
Fig. 25. Dorsal; Fig. 26. Lateral; Fig. 27. Egg sac.

(Fig. 27) are cylindrical, tapering posteriorly, and are very loosely attached to the body slightly posterior from their anterior ends.

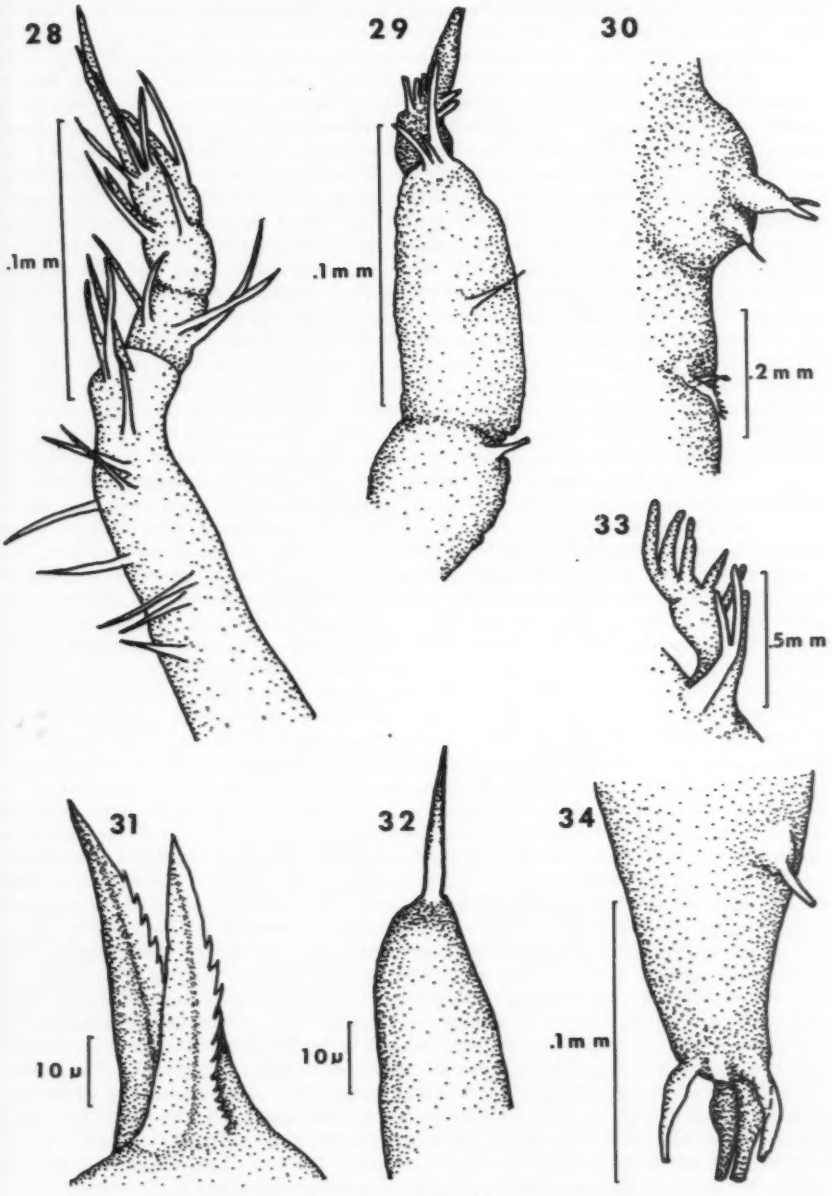
Appendages — The first antenna (Fig. 28) arises from the ventral surface anterior to the lateral cephalic lobe. The proximal portion curves dorsally whereas the distal half extends laterally away from the body so that it is visible in dorsal view. Progressing distally the three segments are armed with 11, 4, and 10 well-developed setae. In Fig. 28 the proximal portion of the second segment is curving strongly out of the focal plane and, hence, appears shortened. The second antenna (Fig. 29) is located immediately posterior to the first and is composed of three segments with a possible fourth masked by the apical setae. The first segment bears a stout seta on its anterolateral margin and is well demarcated. The second segment is armed with a slender midventral seta as well as three well-developed setae on its distal end. The third segment arises from the dorsal portion of the second and terminates with a large inflated seta flanked by five short, robust setae. These setae are often damaged (Fig. 29); this may be a natural condition or a result of fixation and handling. The mouth parts can be extended from the buccal capsule (Fig. 30) or completely withdrawn (Fig. 26). The second maxilla, which carries no evidence of segmentation, terminates in two cutting blades (Fig. 31) each armed with rows of denticles. A long smooth spine (Fig. 32) curving slightly ventrally projects from the tip of the maxilliped, which is also unsegmented. The mandible and first maxilla are apparently absent. Two structures (Fig. 30 and 33), possibly remnants of thoracic legs, are located on the ventrolateral surface approximately 0.17 from the buccal capsule. The principal structure consists of six fingerlike outgrowths arranged on the ventral margin of a basal stalk. A secondary outgrowth occurs near the base of the principal structure and bifurcates to produce two additional projections that resemble those on the main growth. The secondary structure originates on or very close to the base of the primary growth. The tip of the caudal furca (Fig. 34) is equipped with four large inflated setae in addition to a single seta arising from a secondary cuticular outgrowth on the ventrolateral surface. These setae were also always observed in a damaged condition.

Measurements — The following measurements are based on 23 specimens. Total length, from anterior extremity to bifurcation of caudal furcae 6.0 (4.6–7.0); length of cephalic segment, from anterior extremity to posterior margin of lateral cephalic lobes, 1.8 (1.2–2.2); length of thoracic segment, from cephalic segment to first genital segment, 2.0 (1.4–2.4); length of genitoabdomen, from thoracic segment to bifurcation of caudal furcae, 2.2 (1.5–2.6); egg sacs, based on 9 measurements, 2.2 × 0.6 (1.9–2.8 × 0.5–0.9).

Description of the Male

The general appearance (Fig. 35) closely resembles that of other males of the genus. Following the currently accepted plan (Calman, 1909; Delamare-Deboutteville, 1962; and Noble et al., 1969) the tapered, 11-segmented body may be divided into head, thorax, and abdomen. The head is represented by the anterior portion of segment 1, the thorax by the posterior portion of segment 1 and segments 2–6, the abdomen by segments 7–11. The posterolateral margins of the dorsal surface of the second full thoracic segment are produced into elongated processes that are directed posteriorly then curve dorsolaterally away from the body. The last abdominal segment is divided longitudinally by a median fissure that extends from the posterior margin to the middle of the segment and also bears elongate, unsegmented caudal rami.

Appendages — The five-segmented first antenna (Fig. 36) originates from the anteroventral margin of the head and curves dorsally. The first segment is short; the second segment is equal to the combined length of the distal three segments. Progressing distally the segments are armed with 1, 9, 4, 3, and 6 setae. The setae on the first two segments are very stout and are directed anteriorly. No aesthetes were observed. The apical armature consists of three robust setae flanked by three more slender, shorter setae. The second antenna (Fig. 37) arises immediately posterior to the first antenna and consists of four segments. The first segment is short and broad, curving laterally as it joins the second, which constitutes most of the length of the appendage. The third segment is very short and not well delimited proximally, whereas the fourth narrows abruptly

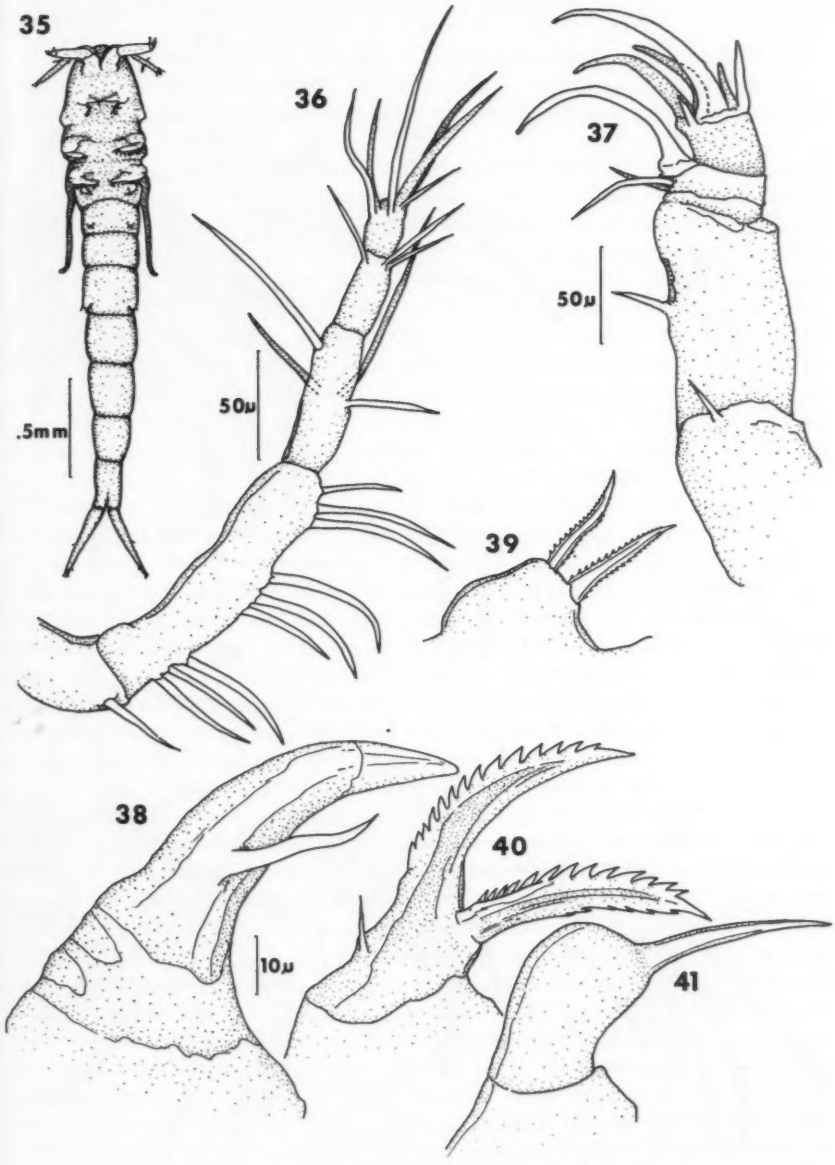


FIGURES 28–34. Female appendages, *C. kyphosus*.

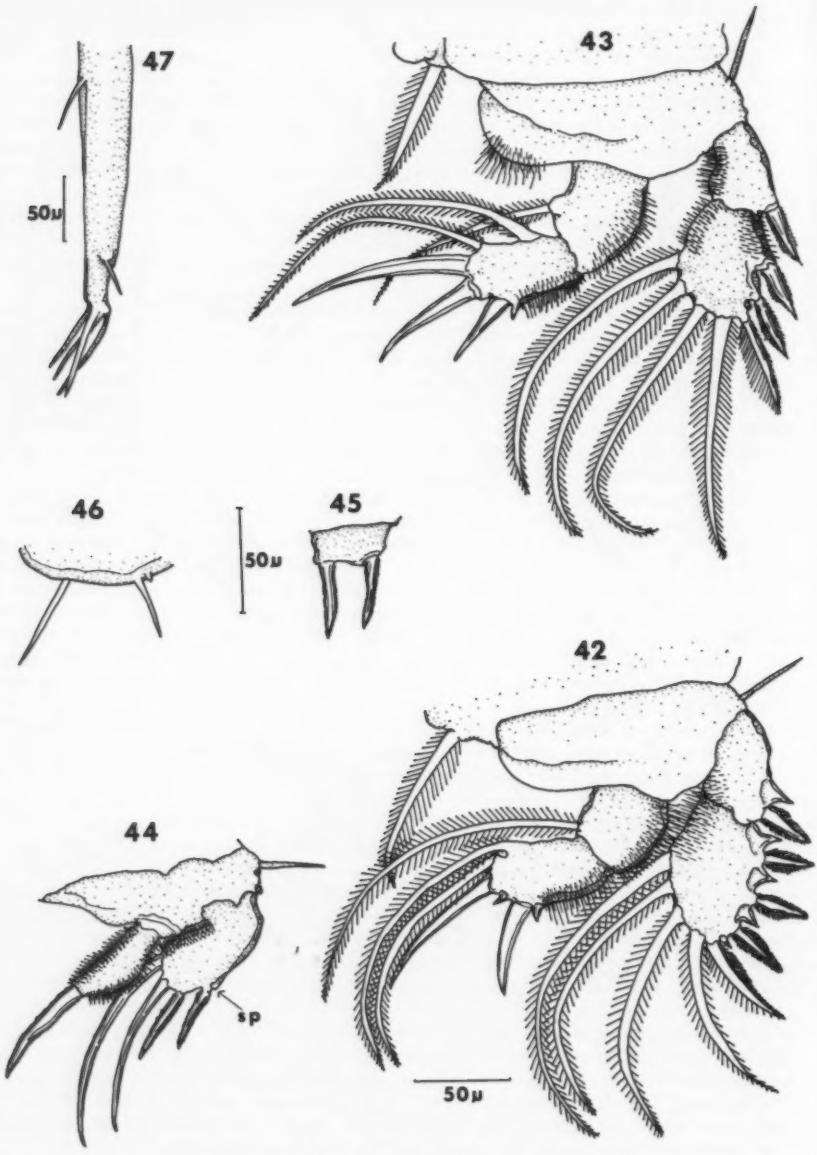
Fig. 28. First antenna, ventral; Fig. 29. Second antenna, ventral; Fig. 30. Buccal capsule, lateral; Fig. 31. Maxillary blades, lateral; Fig. 32. Maxilliped, lateral; Fig. 33. Structures posterior to buccal capsule, medial; Fig. 34. Tip of caudal furca.

and is slightly longer than the third. Progressing distally the armament includes 1, 1, 3, and 6 setae. These may be separated into three types: spinous (such as those on segments 1, 2, and 4), uncinate spinous (the large, well-developed setae on segments 3 and 4), and simple setae (found only on the proximal portion of segment 3). The appendage is held in a position so that the uncinate spinous setae curve anteriorly. The mandibular blade (Fig. 38) arises from a broad base in the middle of the first segment. A single denticle of variable length originates from the posterior surface and tapers to a fine point. The denticle illustrated in Fig. 38 is exceptionally long. The first maxilla (Fig. 39) lies posterior and slightly medial to the mandible and consists of one broadly based segment whose medial margin is appreciably shorter than the lateral margin. Two terminal spines, themselves minutely denticulated, arm the tip. The second maxilla is located posterior to the first maxilla and consists of two segments, the distal segment terminating in maxillary blades (Fig. 40). Well-formed denticles arm the ventral edges of each blade, but the dorsal edge of the ventral blade is smooth and the dorsal edge of the dorsal blade bears only 4–6 fine denticles on its distal half. There is also a slender seta at the base of the ventral blade. The maxilliped (Fig 41) is posterior to the second maxilla and consists of a short broad segment and a terminally rounded segment that gives off a long dorsally curved spine from its medial surface. The four pairs of thoracic legs originate from the ventral surface of the first four free thoracic segments. Dense patches of fine setules are present on the medial surfaces of the exopods and the lateral surfaces of the endopods of the first and second legs. In addition the second leg has a patch of similar setules on the lateral surface of the distal segment of the exopod and another on the posteromedial margin of the basis. The protopod of the first thoracic leg (Fig. 42) is two-segmented and both exopod and endopod are two-jointed. The basal segment of the exopod bears a proximal spinous process and a distal spine on its lateral margin; the spine has a fine hyaline pectinate fringe. (Such structures will be referred to as fringed spines.) The distal segment of the exopod has four spinous processes and three fringed spines arranged alternately along its lateral margin. Slightly distal to the last spinous process is a longer spine that is fringed on one side and plumose on the other. From this point four long plumose setae are arranged palmately on the distal and medial margins of the segment. The basal segment of the endopod bears a long plumose seta on its medial margin; the distal segment has three spinous processes and two large naked spines arranged alternately along the lateral margin and two long plumose setae at the tip and medial margin. The basis is armed with a single slender seta on its lateral edge and the coxa, a long plumose seta on its medial margin. The second thoracic leg (Fig. 43) is similar to the first except that spinous processes are absent from the exopod and it has only two fringed spines along the lateral margin of the distal segment. The spinous processes on the endopod are also reduced, there being only one on the lateral margin of the distal segment. It also differs in that three naked spines are present at the tip. The spinous processes, especially prominent on the first thoracic leg, are simple outgrowths of cuticle whereas the other structures arise from well-defined bases. The third thoracic leg (Fig. 44) is much reduced but still consists of a protopod and an exopod and endopod in which the two segments are not well defined. A slight indication of a spinous process exists on the lateral margin of the exopod followed by two fringed spines and two naked setae. The endopod consists of a single elongate segment terminating in a large fringed spine. The lateral margin of the protopod retains the slender seta of the first and second legs. The fourth thoracic leg (Fig. 45) is even more reduced, consisting of a single segment terminating in two fringed spines, the medial spine being slightly longer than the lateral. In addition one specimen bore a single slender seta that was located slightly lateral to the lateral fringed spine. Whether the lack of this structure in other specimens is a result of individual variation, maturation, or mutilation is not known. The posterolateral corners of the first abdominal segment are produced into small rounded outgrowths (Fig. 35 and 46) that overlap the most posterior portion of that segment. A long lateral seta and a shorter medial seta flanked by a papilla are located on this process. The caudal furca (Fig. 47) is armed with one subterminal and four terminal setae. The base of the dorsomedial terminal seta is often produced into a rounded knob along its medial margin. There is also one seta on the lateral surface in the middle of the furca.

Measurements — The following measurements are based on 10 specimens. Total length, not including caudal furcae, 2.3 (2.0–2.6), maximum width 0.42 (0.40–0.45).



FIGURES 35–41. Male, and male appendages, *C. kyphosus*.
Fig. 35. Male, ventral; Fig. 36. First antenna, dorsal; Fig. 37. Second antenna, ventral; Fig. 38. Mandibular blade, posterior; Fig. 39. First maxilla, posterior; Fig. 40. Maxillary blades, posterior; Fig. 41. Maxilliped, posterior. Same scale for Fig. 38–41.



FIGURES 42–47. Male appendages, *C. kyphosus*.

Fig. 42. First thoracic leg, ventral; Fig. 43. Second thoracic leg, ventral; Fig. 44. Third thoracic leg, ventral (rudimentary spinous process indicated by arrow); Fig. 45. Fourth thoracic leg, ventral; Fig. 46. Process on first abdominal segment, ventral; Fig. 47. Caudal furca, dorsal. Same scale for Fig. 42–44.

Discussion

The first report of any species of *Colobomatus* from the eastern Pacific coast was by Noble et al. (1969) who described *C. embiotocae* from surfperches. The present report describes the second species of the genus to be discovered in the eastern Pacific.

The female *C. kyphosus* can be distinguished from all other species of *Colobomatus* except *C. pagelli* (Richiardi, 1877) and *C. sieboldi* (Richiardi, 1877) in that genital lobes are absent. *Colobomatus kyphosus* is readily distinguished from these latter species by the relatively large median cephalic lobe and the well-developed thoracic lobes. Unique characteristics of *C. kyphosus* are the broad winglike lateral thoracic lobes, the large biramous dorsal thoracic lobe, the structures immediately posterior to the buccal capsule, the presence of second antennae, and the apparent absence of mandibles and first maxilla.

Including *C. kyphosus*, males of six species of the genus have been described: *C. agassizi* (Richiardi, 1877), *C. sciaenae* (Richiardi, 1876), *C. embiotocae*, *C. grubei* (Richiardi, 1877), *C. canthari* (Richiardi) Delamare-Deboutteville and Nunes, 1952. The general morphology of all males described is similar and only the descriptions of *C. sciaenae* and *C. embiotocae* are adequate for detailed comparisons. Segmentation of the appendages and details of the mouthparts reveal the similarities between *C. kyphosus* and *C. embiotocae*. With the exception of the extremely long second segment of the first antenna this appendage resembles the six-segmented antenna of *C. embiotocae*. In addition the distal two segments of the antennae are similarly armed with three and six setae. The second antennae are also similar in having a broad basal segment, a long second segment, and uncinate setae on the distal segments. Differences in terminal segmentation may be due to different interpretations of segmental lines (between the second and third segment), which are very obscure in *C. kyphosus*. The mandible and second maxilla are similar to those of *C. embiotocae* except for the mandibular denticle, the seta at the base of the ventral maxillary blade, and the well-defined denticles that arm the maxillary blades of *C. kyphosus*. Hairlike setae were described as arming the maxillary blades of *C. embiotocae*. The first maxilla of *C. kyphosus* are large, well-formed appendages, the first to be described in the genus. The maxillipeds are also basically similar to those of *C. embiotocae* with the exception that the terminal armament is a strong spine in *C. kyphosus* whereas a terminal seta was described in *C. embiotocae*. The first thoracic legs of the two species follow a similar pattern with the differences that the short spinous processes on the lateral margins of the exopod and endopod are not reported in *C. embiotocae*, and the exopod possesses four and the endopod two plumose setae instead of the three and four respectively described in *C. embiotocae*. With the exception of a single spinous process on the endopod and four instead of three plumose setae on the exopod the second thoracic leg also resembles that of *C. embiotocae*. The fringed spines of *C. kyphosus* are all described as naked setae in *C. embiotocae*. The setae on the lateral margins of the basis of the first, second, and third thoracic legs have not been previously reported. *Colobomatus kyphosus* shares

with *C. grubei* and *C. agassizi* the characteristic of possessing four pairs of thoracic legs but descriptions of these species are inadequate. Two setae on each side of the first abdominal segment were described in *C. embiotocae* but the medial papillae present in *C. kyphosus* were not mentioned. The caudal furcae of *C. kyphosus* are similar to those of *C. embiotocae*, *C. canthari*, and *C. grubei* in being composed of one segment. *Colobomatus sciaenae* is the only species described to have furcae of two segments.

In comparing the mouthparts of the female and male it seems logical to assume that the mandibles and first maxillae of the female are absent. What are surely the second maxillae and maxillipeds in the male (Fig. 40–41) correspond to similar structures in the female (Fig. 31–32). Also in support of this hypothesis is the location (Fig. 30), medial and posterior, of these structures in the female's buccal capsule. Although much more obscure in the female, the general pattern of segmentation and armament of the first and second antennae are similar to that of the male. The armament of the caudal furca is also very similar. The only difference is the presence of lateral seta in the middle of the furca of the male; this is absent in the female.

A problem arises in the description of the dorsal thoracic outgrowth. Within the genus there can be seen a tendency for the bases of the thoracic lobes to merge and produce a single structure with extended tips. Such structures can be seen on *C. agassizi* and *C. embiotocae*. Conversely, biramous thoracic lobes are present in *C. sciaenae*, *C. denticis* (Richiardi, 1877), and *C. benazzii* Delamare et Ruivo, 1952. Since the base of the structure on *C. kyphosus* extends well away from the body before bifurcating and no signs of fusion are evident, it is described as biramous.

Colobomatus kyphosus was recovered from 56.1% of the fish examined. One hundred and eleven females and 12 males were collected; of these, 24 females were found in the dentary canals making them a secondary habitat of considerable importance. To my knowledge the dentary canals have not been previously recorded as a habitat for any species of copepod. Intensity of infection averaged 3.3 (1–15) parasites per fish. The heaviest infection consisted of 13 females and 2 males. In one infection only egg sacs were found. In many others, dead specimens in very poor condition with only a cuticular shell remaining were found within the canals; in the latter cases egg sacs were missing.

Chondracanthus pinguis Wilson, 1912

Habitat — On and adjacent to gill arches.

Incidence of infection by this species was 54.5%, the average intensity being 5.3 (1–31). Of the 192 specimens collected 148 were juveniles as denoted by their small size and absence of egg sacs. Some of the juveniles were found loosely attached to the gill filaments but this habitat is not occupied by the adults.

Sarcotaces arcticus Collett, 1874

Habitat — Cyst in anal region of abdominal cavity.

Of the three infected fish, each harboured a single female of this species yielding an incidence of infection of 4.5%. In two instances the cysts were very old and contained hardened black material. This report constitutes a new host record for *S. arcticus*.

Clavella parva Wilson, 1912

Habitat — Fins.

A total of 64 specimens was found to infect 36.4% of the fish examined. Average intensity of infection was 2.7 (1–10). Of the 64 specimens recovered 30 were removed from the caudal fin, 10 from the anal fin, 4 from the pelvic fin, and 20 from the soft-rayed portion of the dorsal fin.

Brachiella robusta (Wilson, 1912) Kabata, 1970

Habitat — Gill rakers.

Of the fish examined 69.7% were infected with this species. A total of 206 specimens was collected, predominantly from the gill rakers of the first gill arch. Average intensity of infection was 4.5 (1–21). In two instances this species was found attached to the dorsal surface of the buccal cavity but this habitat is rarely utilized.

DISCUSSION

The present study indicates that of the six species of copepods found to parasitize *S. alutus*, *Chondracanthus triventricosus* and *Brachiella robusta* are most prevalent. *Colobomatus kyphosus* and *Chondracanthus pinguis* are also frequently encountered, occurring in over 50% of the fish examined. *Clavella parva* is less abundant and seems to utilize all fins except the pectorals.

Although *Sarcotaces arcticus* proved to be the least prevalent, it is frequently reported by fishermen and fisheries biologists of the area. These reports are probably prompted by the large size and prominent appearance of this copepod whereas other inconspicuous parasites, although apparently more common, are not noticed. The low incidence of *S. arcticus* might also be attributed to the uniform size of fish examined in this study. The two new species, *Ch. triventricosus* and *C. kyphosus*, probably have not been reported previously since their locations in the host are infrequently examined.

ACKNOWLEDGMENTS

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Distribution and Abundance of Tubificid (Oligochaeta) Species in Toronto Harbour, Lake Ontario

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BRINKHURST, R. O. 1970. Distribution and abundance of tubificid (Oligochaeta) species in Toronto Harbour, Lake Ontario. J. Fish. Res. Bd. Canada 27: 1961–1969.

The tubificid oligochaetes build up populations in excess of 200,000/m² in Toronto Harbour. Pollution-tolerant species in great abundance are located around the mouth of the Don River, whereas small numbers and some less tolerant species are found near the island shores. There is no evidence of diurnal rhythms in the vertical distribution of the worms in the sediment. Dry weights for tubificids are about 16% of the wet weight for fresh-starved worms. The ash content averaged 34% of the dry weight for worms with a gut full of mud, but 11% for worms clear of gut contents.

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INTRODUCTION

TORONTO HARBOUR, Lake Ontario, receives a considerable load of raw, partially treated and treated sewage as well as other organic and inorganic pollutants from the Don River, the storm water overflows of the City of Toronto, large and small ships, summer homes on the Toronto islands, and surrounding industries. A detailed study of the relation between the worm population and certain constituents of the sediment is currently being undertaken, following the pilot study of Brinkhurst and Chua (1969). In this paper the distribution and abundance of species present, and some data on dry weight determinations, are discussed. One set of samples was taken every 4 hr for a period of 20 hr to check for diurnal changes in vertical distribution of the worms.

MATERIALS AND METHODS

The survey of worms in Toronto Harbour was carried out during May–July 1969, the sampling stations being based on a grid plan. Four samples were taken at each of the 43 stations with a KB corer (5-cm diameter) described elsewhere (Brinkhurst et al., 1969). Samples were screened through narrow mesh screens (0.2-mm opening) and worms were picked using stereo microscopes. Two of the samples were used to determine the dry weight of worms (plus gut contents) at each station. Eight of these samples were used to determine the average dry weight of a worm. This value was used to convert dry weight values to standing stock estimates. The other two samples were used to determine the identity and abundance of each species present. Average values of dry weight for worms minus gut contents for each species were used to obtain calculated dry weight estimates. Some additional data from continuous studies at Hanlan's Point are used in addition to the survey data.

Total coliform counts and Biochemical Oxygen Demand (BOD) and dissolved oxygen values cited were obtained by Ontario Water Resources Commission (OWRC) personnel in 1967 in an

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extensive survey of Toronto Harbour. The stations used by the OWRC covered the harbour but did not coincide with those used here. Total nitrogen and phosphorus values were determined for the Don River discharge in 1969 by OWRC analysts.

Study Area

Toronto Harbour has a surface area of 373 ha and a maximum depth of 12.5 m. The Don River enters the northeast corner of the harbour via the Keating Channel. The harbour is connected to Lake Ontario via two narrow channels (marked by arrows in Fig. 1) known simply

FIG. 1. Sampling stations in Toronto Harbour, survey dated May–July 1969.

as the East and the West gaps. The northern and eastern sides are embanked and are used as docks, and the banks are covered with warehouses and industries. The south and west shores are formed by the Toronto islands, between which run a series of lagoons and channels. The islands are mostly used as parkland, with a few residents still occupying the southeast corner. A number of sailing clubs and a small airport form part of the recreational facilities.

The two major sewage works on the Toronto waterfront discharge their effluents on either side of the harbour in Ashridges Bay and Humber Bay. Storm sewers discharge into the harbour along the northern wall, and the Don River carries a heavy load of organic and industrial wastes.

RESULTS

There are a number of ways of looking at the data derived from this survey. The overall average of numbers, weights, and relative abundance is valuable, but where there are marked differences from site to site some further elaboration is required. To attempt to display the data for each station would require considerable space, and so a compromise solution is adopted. The harbour was divided into zones with respect to certain characteristics, particularly in terms of estimates of the total number of worms observed. Average values for these zones have been calculated.

The distribution of tubificids as a group is illustrated in Fig. 2. Four zones have been identified, with populations varying from greater than 125,000 to less than 25,000/m². The most abundant species throughout the study area are *Tubifex tubifex* (Müller), *Limnodrilus hoffmeisteri* Claparède, and

FIG. 2. Abundance (no./m²) of tubificids in Toronto Harbour. Zone 1 (solid), > 125,000; Zone 2, 75,000–125,000; zone 3, 25,000–75,000; zone 4, > 25,000.

Peloscolex multisetosus (Smith), and the percentage of the total that each represents is illustrated (again in zones) in Fig. 3–5. Other species were present but relatively scarce. *Limnodrilus udekemianus* Claparède was fairly abundant in zone 1 (an average of about 10,000/m², less abundant in a narrow zone roughly equivalent to zone 2 (2500/m²), but across the bulk of the study

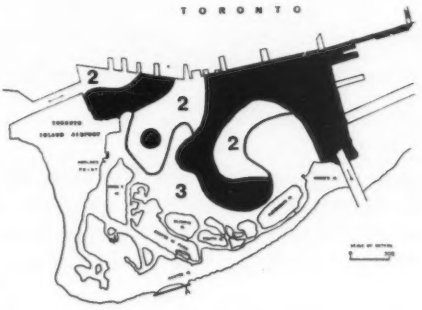

FIG. 3. Abundance of *Tubifex tubifex* in Toronto Harbour as percentage of all tubificids. Zone 1 (solid), > 50%; zone 2, 25–50%; zone 3, < 25%.

FIG. 4. Abundance of *Limnodrilus hoffmeisteri* in Toronto Harbour as percentage of all tubificids. Zone 1 (solid), >50%; zone 2, 25–50%, zone 3, <25%.

area (Fig. 6) a single specimen per core was obtained (which is equivalent to a little over 500/m²). Two other *Limnodrilus* species (*L. cervix* Brinkhurst and *L. claparedeanus* Ratzel) were generally distributed, with a slight concentration along the north shore and particularly in the Don River mouth so far

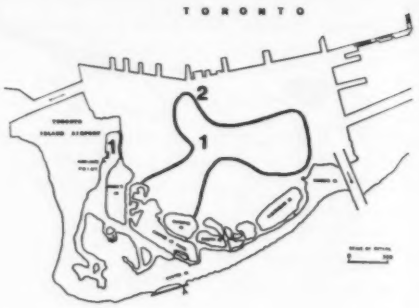

FIG. 5. Abundance of *Peloscolex multisetosus* in Toronto Harbour as percentage of all tubificids. Zone 1, >25%; zone 2, <25%.

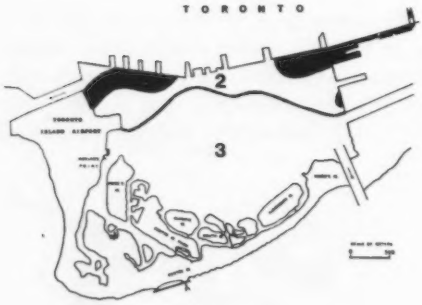

FIG. 6. Abundance (no./m²) of *Limnodrilus udekemianus* in Toronto Harbour. Zone 1 (solid), >5000 (mean, 9500); zone 2, 1000–5000 (mean, 2500); zone 3, 0–500 (mean, 360).

as *L. cervix* is concerned. However, all three *Limnodrilus* species (other than *L. hoffmeisteri*) account for less than 6% of the total overall samples. The average abundance of these species in the study area was estimated to be under 5000/m² (Table 1). The three other species concerned were *Aulodrilus pluriseta*

TABLE 1. Estimates of the abundance of tubificid species and their percent representation derived from averaged data on two samples per station.

	Species					
	Tubifex tubifex	*Limnodrilus hoffmeisteri*	*Peloscolex multisetosus*	Other *Limnodrilus* spp.	Other species	Total
No. (*thousands/m²*)	39.0	25.8	13.0	4.7	1.3	83.8
%	46.5	30.9	15.5	5.6	1.5	100

(Piguet), *Potamothrix vejdovskyi* (Hrabe), and *Potamothrix hammoniensis* (Michaelsen). All three were restricted to the south or island shores of the study area, but although as many as 9000/m² of the former were found at one station (Fig. 7), only a single specimen per core of *P. vejdovskyi* was reported (eight per core at a single station), with only a single specimen of *P. hammoniensis* being found throughout the study. The six minor species accounted for about 7% of the whole.

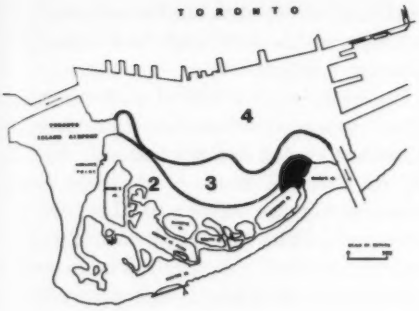

FIG. 7. Abundance (no./m²) of *Aulodrilus pluriseta* and *Potamothrix vejdovskyi* in Toronto Harbour. *A. pluriseta*: zone 1 (solid), 9250; zone 2, 750–3750 (mean, 2000); zone 3, 250–750 (mean, 350); zone 4, absent. *P. vejdovskyi*: zones 1 and 2 present (mean, 800); zones 3 and 4, absent.

Data for the three most abundant species in four zones of Fig. 2 are presented in Table 2. The average dry weights for the tubificid species are 0.2 mg for *T. tubifex*, 0.3 mg for *L. hoffmeisteri*, and 0.1 mg for *P. multisetosus*. These values were established using thousands of worms held in the laboratory until the guts were empty. The dry weight of these worms is about 16% of

TABLE 2. Data on the distribution and abundance of the three most abundant tubificids (average number/m², percent, and dry weight in g/m²) for each of the four zones identified in Fig. 1, and for Hanlan's Point, Toronto Harbour. T = *Tubifex tubifex*; L = *Limnodrilus hoffmeisteri*; P = *Peloscolex multisetosus*.

		Zone				
		1	2	3	4	Hanlan's Point
No. stations		8	11	22	2	1
Avg no./m² (*thousands*)	T	115	55	18	3	12
calculated from dry weight plus	L	58	33	21	8	16
gut content	P	24	16	12	12	2
		197	104	51	23	30
		194	106	59	19	–
%	T	58	53	36	14	40
	L	30	31	41	33	53
	P	12	16	23	52	7
Avg dry weight (*g/m²*)	T	22.9	11.0	3.7	0.7	2.4
calculated for worms minus	L	17.5	9.8	6.3	2.4	4.9
gut content	P	2.3	1.6	1.1	1.2	0.2
		42.7	22.4	11.1	4.3	7.5
Avg dry weight (*g/m²*) of worms plus gut contents (all species)		98.5	48.0	26.5	9.0	–
Ratio dry weight plus gut content/dry weight without gut content		2.3:1	2.1:1	2.4:1	2.1:1	–

the wet weight, and the ash constitutes 11% of the dry weight. The ash content of field-caught worms with the gut contents present was 34% of the dry weight, which was 0.47 mg per worm (averaged for 1381 worms).

The coliform values for Toronto Harbour by the OWRC, obtained in December 1967, fall roughly into two major categories. North of a line joining the east and west channels that connect the harbour with Lake Ontario (Fig. 1) the total coliform count is in excess of 2400/100 ml, south of that line most values lie in excess of 1000/100 ml. The overall average value was 2220/100 ml. Of the 17 values (36% of samples) in excess of 2400/100 ml the average was 4270. Eighty percent of values were in excess of 1000. The BOD values in the harbour were low (1–2 ppm) and dissolved oxygen was high (>70% saturation). The total nitrogen and total phosphorus input to the system via the Don River has been calculated as 830 and 285 tons for the year 1969 (data from OWRC water quality analyses).

The results obtained from taking samples of worms at 4-hr intervals throughout a diurnal cycle are presented in Table 3.

TABLE 3. Vertical distribution of worms in sediment during one diurnal period. Numbers are given per core sample; numbers per square meter are approximately 500 times greater.

Depth in core (*cm*)	Time					
	2 AM	6 AM	10 AM	2 PM	6 PM	10 PM
0–2	47	91	56	10	60	41
2–4	14	40	28	14	36	33
4–6	7	21	9	11	11	12
6–8	2	6	7	4	2	4
8–10	0	4	4	1	5	2
10–12	12	2	0	2	1	5

DISCUSSION

The distribution of tubificids in relation to pollution has been discussed several times, and the subject was reviewed by Brinkhurst (1966). The situation in the St. Lawrence and Great Lakes was reviewed by Brinkhurst (1970) and Brinkhurst et al. (1969).

The results of this survey confirm the view that the tubificids can be used as indicators of organic pollution, but that attention must be focused on the relative abundance of worms and other benthic organisms, as well as the relative abundance of the worm species themselves, rather than on the mere presence of certain species. *Tubifex tubifex* and *L. hoffmeisteri* in great abundance and in the absence of other benthic organisms, including other tubificid species, indicate gross organic pollution. Where conditions are less extreme, *L. hoffmeisteri* is often the most abundant species, but other *Limnodrilus* species are found, together with *P. multisetosus* in the Great Lakes. The diversity

of species increases with the addition of *Aulodrilus* and *Potamothrix* species in regions best described as eutrophic rather than organically polluted, but *L. hoffmeisteri* is still a major component of the worm population. *Tubifex tubifex* is not an important component of the worm fauna of eutrophic situations however, although it reappears in some numbers where worm populations are low in mesotrophic and oligotrophic conditions. I have commented on this distribution (e.g., Brinkhurst et al., 1968), and it can be confirmed by reference to three independent surveys in the Great Lakes alone. Veal and Osmond (1968) found *T. tubifex* in all three basins of Lake Erie, but although it was found at 24% and 38% of stations in the east and central basins, respectively, it was found at only 7% of stations in the western basin. Hiltunen (1969a) also found *T. tubifex* or *Ilyodrilus templetoni*, or both, in small numbers in some of the samples from western Lake Erie, but thought their distribution of no significance. My records concerning this same material, which was studied by Hiltunen in my laboratory, show a small aggregation of *T. tubifex* in the mouth of the Detroit River, where conditions favoured the development of massive populations of *Limnodrilus* species.

In Hamilton Bay, *T. tubifex* was found at stations where the water was less than 12 m deep off the industrial shore, but not at other shallow-water stations. It became increasingly abundant in relation to depth in the deep central depression, where it constituted as much as 57% of the oligochaete population. The species was absent from nonindustrial shallow-water stations within the bay as well as the open lake immediately outside the bay, where the influence of the polluted bay water can be demonstrated. However, out in the open lake the species was again numerous in relation to other oligochaetes. Hiltunen (1969b) found *T. tubifex* in small numbers at several stations in Lake Ontario, the largest number of positively identified specimens being found off Toronto Harbour, possibly in the area affected by the dumping of material dredged from the harbour in the vicinity of the Don River.

In Toronto Harbour, *T. tubifex* dominates the worm population of both zones 1 and 2, and in the Don River itself this species and *L. hoffmeisteri* make up the entire benthos and are present in great abundance. The distribution of the zones of abundance of worms in general and the distribution of the other tubificid species clearly demonstrate that the Don River is the major source of organic pollution to the harbour and that, despite considerable improvement in quality in recent years, it still has a massive impact on its quality. This is supported by the coliform values reported, although the single set of BOD values for the harbour are low, and the oxygen values high. The tubificids can be used to detect major sources of organic pollution, even in situations where the abundance of worms is so high across the whole study area that more superficial surveys might merely suggest that the whole area is polluted.

Many of the most important benthic organisms in heavily polluted situations are widely distributed, cosmopolitan, adaptable species like *L. hoffmeisteri*, or are species that seem to thrive in marginal situations where competition from specialized species with narrow tolerances (like *Potamothrix*

or *Aulodrilus* species) is probably minimal. Therefore the classification of species in relation to their pollution tolerance according to the Saprobien System so widely used by European pollution biologists should be avoided. The definition of *T. tubifex* as a saprophilic species would make it difficult to explain its presence in Lake Superior or in the many unpolluted places in which it can be found. The cosmopolitan nature of *L. hoffmeisteri* in terms of the habitats in which it may be found would make it impossible to classify in such a scheme, but it may be used as a sensitive indicator of local conditions despite this. Indeed, one of the attributes of a useful indicator is that it be geographically and ecologically cosmopolitan.

The patterns of distribution of the tubificid species established by this survey are adequate for the detection of pollution, but more detailed ecological studies would require more accurate determinations of relative abundance based on six cores per site rather than two, and temporal changes would have to be taken into account. Hence, no further analysis of the data is attempted here. The survey is intended as a reference point for further detailed studies in Toronto Harbour in which the sampling programme can be established in relation to the distributions observed here. The conversion of dry weight values to numbers (using the average value of 0.47 mg/worm plus gut contents) resulted in standing stock estimates close to those obtained by counting. Dry weight estimates for identified animals held in the lab until the guts were emptied were used to calculate the biomass of worms in the field. The values obtained were slightly less than half the values obtained by measurement, the consistent difference being attributable to the gut contents. The good agreement between calculated and observed values in each instance serves to confirm the reliability of the values obtained.

The vertical distribution of tubificids has been established earlier (Brinkhurst and Kennedy, 1965; Brinkhurst et al., 1969), but no attempt has been made to date to establish the presence of diurnal activity, which might be of importance in studies of the feeding habits of the worms. An additional set of samples was taken at intervals during a single diurnal cycle (Table 3). The number and distribution of worms remained fairly constant throughout, apart from the sample taken at 2 PM in which rather few worms were found near the surface. Reference to four sets of 10 samples taken at the same site on a different occasion (Brinkhurst et al., 1969) showed that this sample could be regarded as aberrant, but in any case there was no significant difference with respect to distribution of worms down the core, and no evidence of movement in either direction could be obtained. These worms have never been reported in the water out of the sediment, and, in over 10 years of laboratory observations, have never been seen to leave the mud.

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Defecation Rate of Three Tubificid Oligochaetes Found in the Sediment of Toronto Harbour, Ontario[1]

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Appleby, A. G., and R. O. Brinkhurst. 1970. Defecation rate of three tubificid oligochaetes found in the sediment of Toronto Harbour, Ontario. J. Fish. Res. Bd. Canada 27: 1971–1982.

The defecation rate of three tubificids is affected by temperature but the effect differs from species to species. Information on defecation rate and weight change at each experimental temperature suggests an optimum temperature range of 14 C for *Peloscolex multisetosus*, 18 C for *Tubifex tubifex*, and greater than 20 C for *Limnodrilus hoffmeisteri*. Downward trends of defecation rate with time were recorded for *P. multisetosus*, but these were probably due to lowered activity prior to the observed mortality. Upward trends in defecation rate with time in *L. hoffmeisteri* may indicate continuing acclimation to experimental conditions beyond their 10- to 14-day duration, or a need to process increasing amounts of less nutritious mud because of selective feeding on the richer fractions at the beginning of the assay period. Data on the relative abundance and distribution of species in Toronto Harbour is used to estimate defecation rates of the tubificid populations in the field.

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INTRODUCTION

Tubificids inhabit muddy or sandy aquatic sediments, both marine and freshwater. They feed by ingesting the sediment, and respire via the posterior end of the body, which waves about in the water for at least part of the time. Feces are expelled in the water and fall onto the surface of the sediment; thus the worms actively irrigate the mud with their burrows and mix the sediment by passing it through the gut. Worms may be found to a depth of 20 cm in sediment but usually concentrate at between 4 and 6 cm from the interface. The rate of mixing sediments may be of importance in promoting chemical exchange between mud and water, and may be sufficient to upset microstratification of sediments (Stockner and Lund, 1970). In addition, estimates of secondary production of worms may be calculated by measuring growth (including reproduction), respiration, and egestion in terms of energy or nitrogen or both, from which it is possible to calculate ingestion and to obtain estimates of assimilation efficiency. A study of defecation rate in relation to temperature was carried out by Wachs (1967), using *Tubifex tubifex* (Müller, 1774). Brinkhurst (1970) showed that this species, together with *Limnodrilus hoffmeisteri*

[1]Based on a thesis submitted by A.G.A. in partial fulfillment of the requirements for the degree of Master of Science from the University of Toronto, Toronto, Ont.

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Claparède, 1862, and *Peloscolex multisetosus* (Smith, 1900) dominated the benthos of the polluted Toronto Harbour. The defecation rate of these species in relation to temperature was investigated as part of a detailed study of this worm association and its interaction with the sediment and associated microflora.

METHODS

Worms were collected from Hanlan's Point, Toronto Harbour (Brinkhurst, 1970), with a 15 cm² Ekman grab. Samples were washed through a 0.595-mm screen either in the field or in the laboratory. Mud used in the experiments was stored at 4 C until required. It was then screened (0.42 mm) and allowed to settle overnight, after which the supernatant was removed and the mud was thoroughly stirred.

Three tubificid species were used in the experiment, *T. tubifex*, *L. hoffmeisteri*, and *P. multisetosus*. Worms were picked manually from screened samples, or were allowed to move actively from a screen loaded with drying sediment set under a lamp into a shallow pan of water beneath. Stock groups of each species were maintained, and an attempt was made to select healthy specimens for each experiment. Experimental animals were kept separate for 3 days at the temperature to be used for each particular experiment. Worms were carefully washed with a pasteur pipette and then wet-weighed on a Mettler H2 OT balance in a saturated atmosphere. Three weighings of groups of 10 worms were made, and the average value was used in further calculations.

The weighed worms were placed in a modified version of the apparatus first used by Alsterberg (1922) (Fig. 1). The surface of the mud was covered with a layer of wet cotton, and then

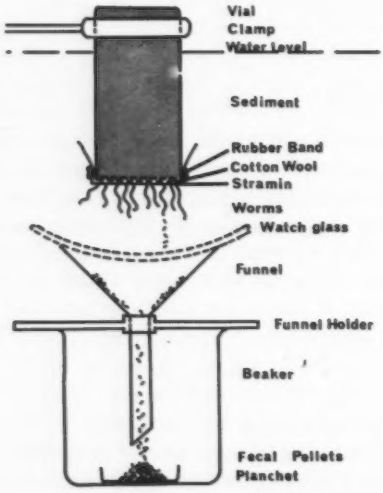

FIG. 1. Apparatus used in inverted worm technique.

a piece of coarse sackclothlike material (stramin), which had been thoroughly washed. Vials were maintained in the normal orientation for 1 day, after which they were inverted.

The vials were checked in order to determine the survival of the worms by observing the number of protruding tails. After a day in the inverted position, the collecting apparatus was introduced to the aquarium. The watch glass was placed in position every time the vials were moved to avoid collecting mud shaken loose from them. The planchets, made from aluminum foil, were retrieved from the collecting apparatus with forceps, and the superficial water was

removed with a pipette. New, labelled planchets were placed in the apparatus, which was replaced beneath the worms, as each vial was used a number of times with samples being taken every 24 hr. Sometimes the setup was left for 3 days in order to evaluate the effect of disturbance on the worms.

Fecal samples were dried overnight at 60–65 C and then desiccated for at least 6 hr at room temperature. Dry weights were obtained with a Cahn gram electrobalance. Worms were wet-weighed after recovery, and again after 3 days during which they were allowed to empty their guts. Dry weights were obtained in exactly the same manner as for the feces.

Experiments (Table 1) were carried out at four temperature ranges (at or about 4, 13, 18, and 21 C), the whole set of vials in their aquaria being maintained in environmental rooms. Temperature, *p*H, and oxygen conditions were monitored. The water content of the mud used in each experiment was also estimated.

LE 1. Details of environmental conditions and duration of experiments (T = *T. tubifex*, L = *L. hoffmeisteri*, P = *P. multisetosus*).

eriment	Temp (*C*)	Species	Replicates	Duration (*days*)	Oxygen (% *saturation*)	*p*H	% water in mud (*by weight*)	Bacterial population (*colonies/g dry mud*) × 10^3
1A	3.7	T	2	15	86–90	7.7	55	4.83
		L	2					
		P	2					
1B	3.5	T	2	19	86–94	7.8	34	12.2
		L	2					
		P	2					
2A	12.4–12.6	T	2	13.5	95–97	7.9	41	6.54
		L	4					
2B	13.9–14.0	T	2	18.5	95–99	7.9	27	10.9
		P	4					
2C	13.6	T	2	9	97–98	7.9	45	6.87
		L	2					
		P	2					
3	18.4–18.6	T	2	4	86–94	7.7	27	11.1
		L	2					
		P	2					
4	21.7–21.8	T	2	10.5	84–90	7.8	35	4.06
		L	2					
		P	2					

Sampling was continued for 9–19 days at 4, 13, and 21 C, but the observations from the 5th day on in the experiment at 18 C were rendered invalid by the amount of sediment that fell from the vials during the collecting period. This material was clearly not of fecal origin.

RESULTS

The variations of defecation rate with time are illustrated for all experiments except No. 3, in which the duration was too short for this to be meaningful

(Fig. 2–7). In Fig. 2 and 3, mean temperature and oxygen conditions for the three aquaria are plotted to indicate the relatively slight variation in conditions typical of all such estimates. The plots of defecation rate per milligram dry worm per hour at 4 C in these two figures are variable from interval to interval,

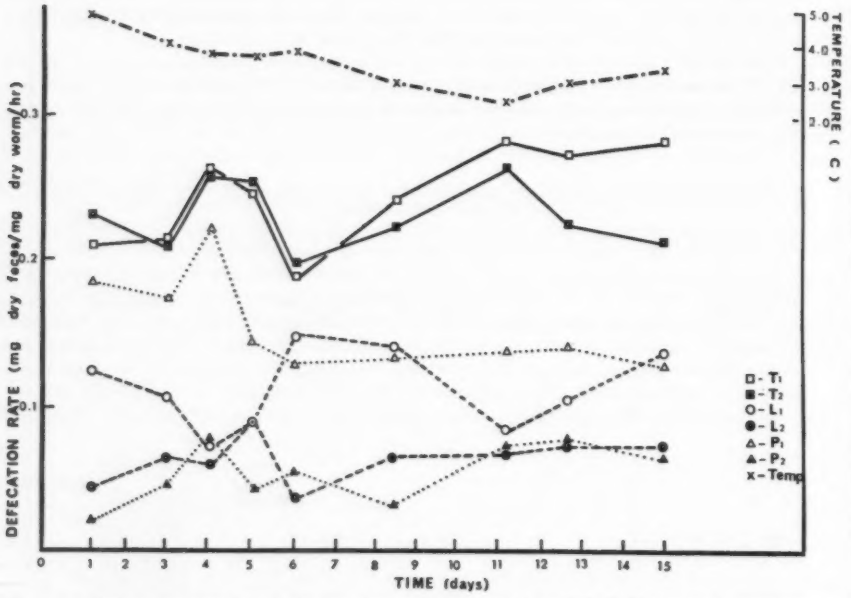

FIG. 2. Defecation rate in relation to time, experiment 1A at 3.7 C. Variation in temperature shown as an insert, mean value for three aquaria. T = *T. tubifex*, L = *L. hoffmeisteri*, P = *P. multisetosus*.

but no consistent rising or falling trends can be detected. The results at the higher temperature around 12–14 C (experiments 2A–C, Fig. 4–6) do show some trends, in particular a steady rise in defecation rate with time for *L. hoffmeisteri*. There is a slight downward trend in defecation rate for *T. tubifex* in 2C (Fig. 6) in which there was mortality, but after initial variation the rates were reasonably steady in the other two runs. The downward trends in the *P. multisetosus* records again reflect mortality among the specimens, as evidenced by the reduction in number of worms eventually recovered in 2B (Fig. 5). Quite steep increases in defecation rate were observed for the first few days for *P. multisetosus* in 2B and for the single set of data obtained in 2C (Fig. 6) in which there was little mortality.

In experiment 4, at 22 C (Fig. 7), the defecation rate of *T. tubifex* fell slightly and that of *L. hoffmeisteri* rose steeply; in neither instance was any significant mortality noted. In *P. multisetosus* the defecation rate was insignificant but although mortality among the worms was high, it was not perhaps sufficient to explain the lack of fecal output.

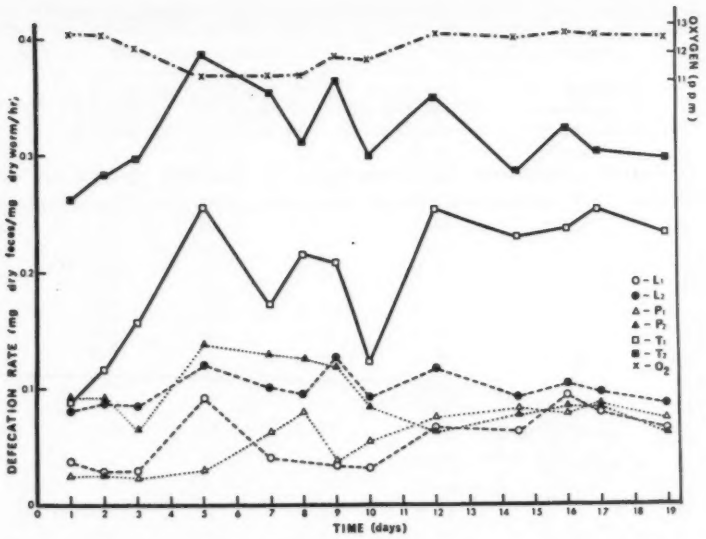

FIG. 3. Defecation rate in relation to time, experiment 1B at 3.5 C. Oxygen value shown as an insert (mean for three aquaria).

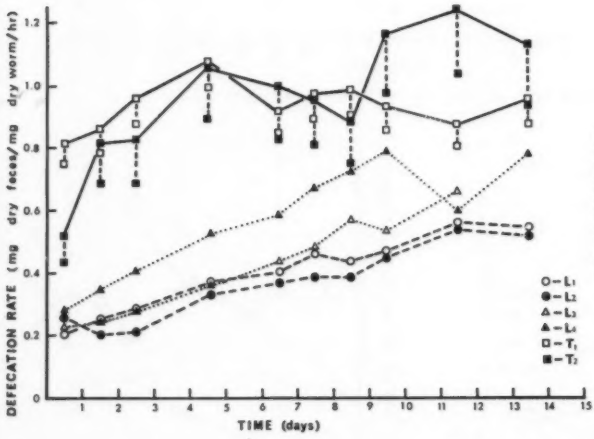

FIG. 4. Defecation rate in relation to time, experiment 2A at 12.5 C. Duplicate symbols linked by dotted line indicate values calculated including and excluding weight of cocoons produced during experiment.

The average defecation rates, the changes in wet weight, and the initial and final number of worms used are itemized in Table 2. The average defecation rates are further illustrated in Fig. 8. The changes of weight observed throughout the study are illustrated in Fig. 9. The percentage of water in the final wet weight of worms was calculated to aid in evaluating any effect of temperature or percent water in the sediment on the wet weight of worms as follows:

Species	Approximate temperature of experiment (C)		
	4	13	20
T. tubifex	88.94±1.1	85.95±0.2	84.95±0.1
L. hoffmeisteri	86.14±3.5	84.06±0.3	82.18±1.1
P. multisetosus	88.34±1.4	86.50±2.2	85.56±1.2

Information regarding the abundance of tubificid species in Toronto Harbour was used to estimate annual defecation rates per square meter in the

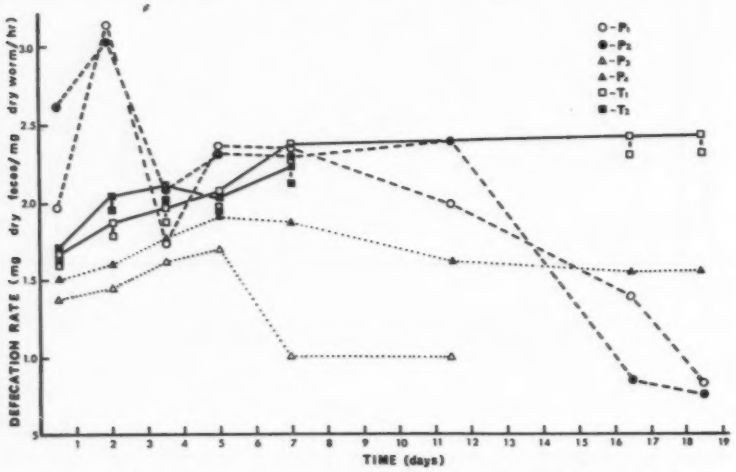

FIG. 5. Defecation rate in relation to time, experiment 2B at 14 C.

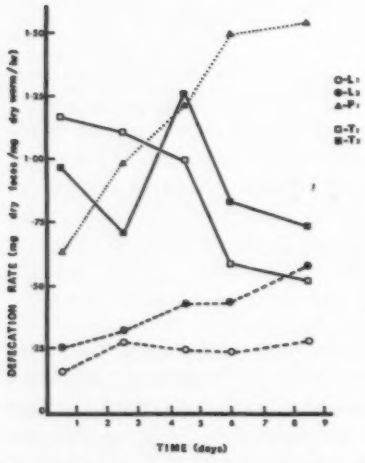

FIG. 6. Defecation rate in relation to time, experiment 2C at 13.6 C.

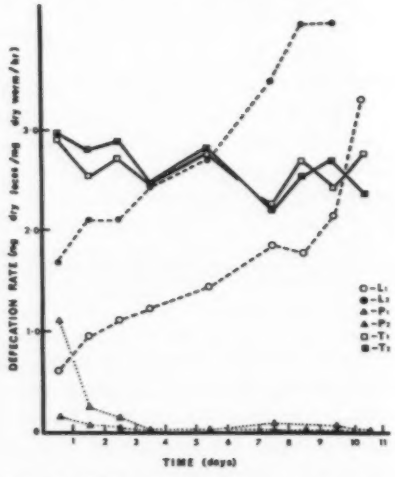

FIG. 7. Defecation rate in relation to time, experiment 4 at 21.7 C.

TABLE 2. Changes in number and weight of worms through the experimental period, and average defecation rate in mg dry feces per mg dry worm per hour for each experiment.

Experiment	Initial no.	Final no.	Initial weight (*mg*)	Final weight (*mg*)	Defecation rate	±SD
1A T_1	10	10	4.46	5.03	0.246	0.035
T_2	10	10	3.23	3.95	0.232	0.022
L_1	10	10	6.30	5.49	0.113	0.026
L_2	10	10	6.27	6.88	0.064	0.014
P_1	10	10	2.45	2.27	0.155	0.030
P_2	10	4	5.18	2.55	0.055	0.02
1B T_1	10	9	5.52	4.15	0.203	0.056
T_2	10	10	3.98	3.46	0.321	0.041
L_1	10	10	6.04	4.36	0.062	0.026
L_2	10	9	4.00	3.34	0.1	0.014
P_1	10	9	1.96	1.16	0.064	0.03
P_2	10	10	2.34	1.76	0.092	0.022
2A T_1	10	8	2.49	3.77[a]	0.938[a]	0.074
T_2	10	9	2.33	3.00[a]	0.967[a]	0.213
L_1	10	10	3.40	2.74	0.4	0.12
L_2	10	10	4.31	4.14	0.368	0.118
L_3	10	10	3.92	3.91	0.46	0.182
L_4	10	9	4.72	3.56	0.551	0.174
2B T_1	10	9	1.95	3.82[a]	2.113[a]	0.3
T_2	10	10	2.44	3.31[a]	2.026[a]	0.2
P_1	10	2	0.59	0.54	1.684	0.694
P_2	10	6	0.91	1.38	2.143	0.829
P_3	10	4	0.58	0.71	1.363	0.321
P_4	10	5	0.43	0.93	1.645	0.184
2C T_1	6	3	2.24	1.25	0.875	0.301
T_2	6	5	2.00	1.71	0.897	0.225
L_1	6	6	8.03	6.61	0.303	0.05
L_2	7	7	5.43	4.64	0.406	0.124
P_1	7	6	0.54	0.47	1.176	0.376
P_2	7	0	0.56	–	–	–
3 T_1	10	6	2.11	3.52[a]	1.985[a]	0.242
T_2	9	7	1.75	3.46[a]	2.393[a]	0.299
L_1	10	10	5.64	7.34	0.369	0.21
L_2	10	9	2.58	4.65	0.705	0.066
P_1	10	0	0.71	–	–	–
P_2	10	5	0.61	0.69	0.263	0.024
4 T_1	10	9	3.41	3.82	2.617	0.21
T_2	10	10	2.80	3.06	2.633	0.266
L_1	10	10	5.66	6.63	1.287	0.445
L_2	10	10	4.31	5.87	2.418	0.622
P_1	10	4	0.56	0.42	0.246	0.388
P_2	10	8	0.62	0.44	0.054	0.06

[a]Cocoons present; excluded from calculation (see Fig. 4, 5).

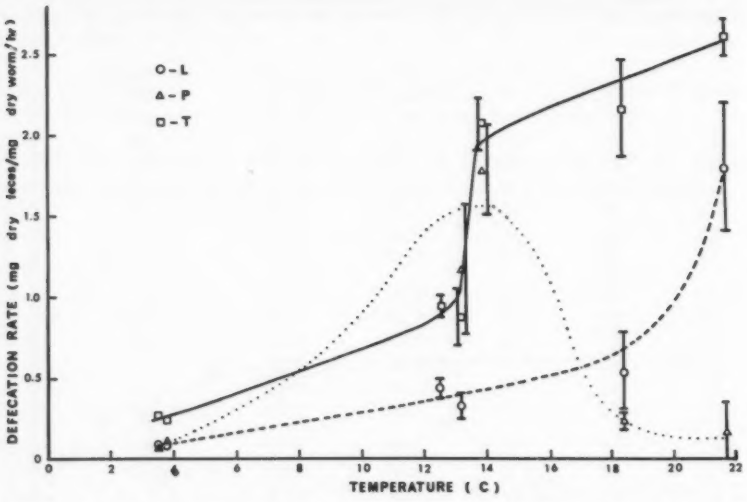

FIG. 8. Defecation rate in relation to temperature. Line fitted by eye to points solely for purposes of discussion.

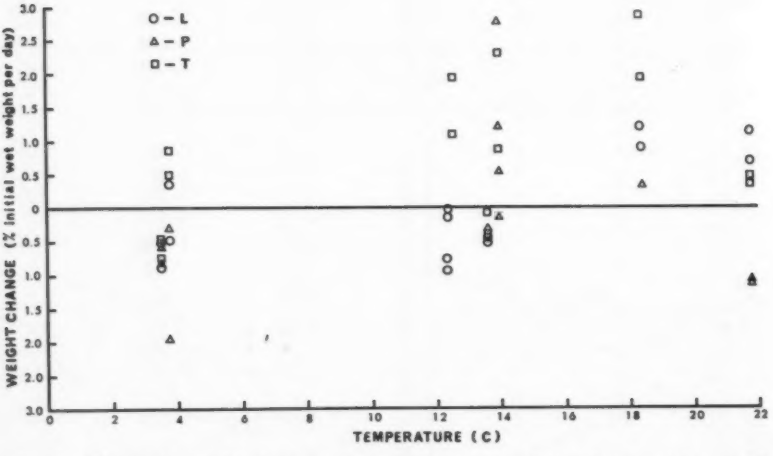

FIG. 9. Change in weight of worms expressed as percent of initial wet weight gained or lost per day during course of each experiment plotted against temperature. Solid line represents no weight change.

four zones and the station at Hanlan's Point that are identified in a separate publication (Brinkhurst, 1970). The defecation rate data obtained from this study was grouped according to temperature as follows:

Species	Temperature range (C) 0–7	7–14	14–21
T. tubifex	0.25	0.92	2.13
L. hoffmeisteri	0.08	0.41	0.54
P. multisetosus	0.09	1.6	0.26

The number of days at which each temperature range applied in the field was obtained from records kept at Hanlan's Point (167, 121, and 77 days, for the ranges shown above) and will have to be revised if data from the open water of the harbour departs seriously from these values. The dry weight of worms was taken to be 15% of the total wet weight for the purposes of these calculations. The resulting data show that the defecation rate in terms of kilograms of feces varies in relation to the abundance of the worm population as might be expected (Table 3).

TABLE 3. Defecation rate in four zones of Toronto Harbour (Brinkhurst, 1970) and at Hanlan's Point. Values in kilograms dry feces per year per square meter (T = *T. tubifex*, L = *L. hoffmeisteri*, P = *P. multisetosus*).

		Zone 1	2	3	4	Hanlan's Point
No. stations		8	11	22	2	1
Avg no. worms per $m^2 \times 10^{-2}$		197	104	51	24	30
Defecation rate per m^2	T	174	84	28	4	18
	L	44	24	16	6	12
	P	12	9	6	7	1
	Σ	230	117	50	18	31

DISCUSSION

A number of factors might affect the defecation rates observed with the experimental setup employed. The inverted position itself was tested by Ivlev (1939) with growth of *T. tubifex* as an index. There was no significant difference in weight gain between worms reared in the inverted and in the normal orientation. The nature of the sediment may well play a role in determining defecation rate, as worms may need to feed less actively on rich organic than sandy sediments. Although there was some variation in the water content of the sediment used, the water content of the worms varied little within experiments despite a trend to loss of water at higher temperatures. Diurnal or irregular fluctuations in feeding activity might go undetected if such variation were not synchronous. Observation suggests that worms are feeding continuously, and there is little evidence of diurnal movements of the worms in sediment (Brinkhurst, 1970). Disturbance of the inverted vials or the undulation of the worms may dislodge a fine drift of sediment that could add to the feces weight; when this occurred, the affected collection was rejected. Trace amounts

might, however, pass unnoticed. The plots of defecation rate against time show that when the worms were undisturbed for 3 days, little effect on the rate was apparent.

More significant was the survival of the worms. Survival in *T. tubifex* and *L. hoffmeisteri* was generally good, but *P. multisetosus* often sustained significant losses as reflected in the downward trend in defecation rates with time.

Loss of worms from the vials was sometimes observed (when the worm dropped into the aquarium), and defecation rates were then calculated on the basis of the number of worms remaining. When the final count of worms was less than the initial count but there had been no recorded loss of a worm, it was assumed that the worm had died in situ, and the defecation rate was calculated on the basis of the average number of worms present throughout the experiment. These adjustments involved *P. multisetosus* for the most part, but the results of duplicate experiments are sufficiently in agreement to suggest that the adjustments had little effect on the result. The course of experiments such as 2B (Fig. 5) might suggest that the mortality of *Peloscolex* in replicates 1 and 2 occurred at about day 11 and at about day 5 for P_3 and P_4.

Steady defecation rate throughout an experiment together with weight gain may be taken as an indication that the worms are thoroughly acclimated to the prevailing experimental conditions. Consistent increases in defecation rate may mean that the worms take longer than 10–14 days to adjust to the conditions, or that the worms need to consume more sediment as their activities reduce the preferred food supply. This assumes that worms can feed selectively, perhaps confirmed in experiments that demonstrate that feces can contain more organic matter than the sediment supply (Poddubnaya, 1961; and personal observation). The increases in defecation rate from the beginning of experiments above 4 C, seen in *Limnodrilus* in particular, might indicate that the worms never reached an equilibrium with the experimental conditions rather than a shortage of preferred food.

Weight gain might be taken as some indication of suitable conditions for the worms. In nine experiments at 4 C, including four with *Peloscolex*, a loss in weight occurred (Fig. 9). Weight increments were recorded once for *L. hoffmeisteri* and twice for *T. tubifex* at this temperature. Weight losses in *P. multisetosus* were recorded at all temperatures (except one at 18 C), but most of the data obtained at 14 C indicated a gain in weight. Weight losses were recorded for *L. hoffmeisteri* in all experiments below 18 C (except one at 4 C), but weight gain was recorded for *T. tubifex* under most conditions.

To some extent the weight-change pattern is reflected in defecation rate. At the lowest temperature studied the defecation rate was minimal, suggesting that the worms remain somewhat inactive during the winter. *Peloscolex multisetosus* seemed to demonstrate maximal activity at intermediate temperatures, where growth was achieved. In both *T. tubifex* and *L. hoffmeisteri* the defecation rate was highest at the highest temperature, with a particularly steep increase in rate in the latter associated with a weight gain about as high as that achieved

at 18 C. In *T. tubifex* the defecation rate is difficult to interpret between 12 and 14 C. There is good agreement between replicates within each experiment, and good agreement between results for experiments 2A and 2C conducted at 12.4–13.6 C, but at 13.9–14.0 C (2B) the defecation rate appears to have doubled. The line drawn more or less through the points in Fig. 8 indicates a steplike increase in defecation at this point, but this is based upon duplicate observations made in a single experiment. The high values obtained at 18 and 22 C indicate that the sort of exponential increase in defecation rate observed in *L. hoffmeisteri* between these temperatures does not occur in *T. tubifex*, or rather that it takes place at a lower temperature.

The defecation rate for both *T. tubifex* and *L. hoffmeisteri* must fall when the worms are approaching temperatures to which they cannot acclimate, and so it is possible to suggest that the data from weight-gain and defecation rate indicate an optimal temperature range of 14 C for *P. multisetosus*, around 18 C for *T. tubifex* and at least greater than 20 C for *L. hoffmeisteri*. The latter is the most tolerant tubificid, being found in practically every habitat, even when exposed to extremes of oxygen deprivation, and temperature and salinity in excess of those tolerated by most tubificids.

No other work can be used for general comparison with these studies. Recalculation of defecation rates observed by Alsterberg (1925) for *T. tubifex* (if identification is acceptable) at 12 C gives an average value of 1.4 mg dry feces per mg dry weight per hr, which agrees with the value for *T. tubifex* obtained here, but is higher than that obtained for *L. hoffmeisteri*.

Ivlev (1939) used silt that was 92.8% water, and the value obtained by recalculating his data is 0.09 mg at 17 C. Ravera (1955) removed worms from sediment and collected the feces deposited in water at 18 C. Production of feces ranges from four times the worm's body weight in Alsterberg's study to only a half or a third of the body weight in Ravera's work with *Psammoryctides barbatus* (Grube) and *Peloscolex ferox* (Eisen). These values approximate closely to those obtained by Ivlev (1939). Wachs (1967) worked with *T. tubifex* and recorded defecation rate by volume, but it is impossible to convert our data to the units used by Wachs. Crude comparisons based on 38% water content of mud and a weight of 1.5 g mud per ml indicate his values are of the same order as ours. What is more significant is the suggestion in Wachs' data of a steep rise in defecation rate around 18 C, producing a stepwise increase somewhat similar to that demonstrated here. The possibility of a threshold effect of temperature in relation to defecation rate should be investigated in detail.

Any attempt to relate defecation rates to estimation of the volume of mud shifted in the field is tenuous. The need to express defecation rates as averages at certain temperature ranges, and the enormous extrapolations involved using a sampler that records numbers per five-hundredth of a meter square (when numbers may reach well over 200 thousand per meter square), imply an enormous magnification of errors. In addition, variation of the water content of mud both horizontally and vertically in the sediment makes it

difficult to employ a consistent average value. The natural variation is not known for Toronto Harbour sediments at present. The values used in converting data from other sources, mentioned above, imply a more or less direct comparison of dry weight of feces in grams to wet volume in milliliters

$$(\text{dry weight in grams} \times \frac{100}{62} \times \frac{1}{1.5} = \text{volume}).$$

Hence the defecation rate in kilograms per square meter per annum can be regarded as equivalent to that in liters. The volume of the layer from 4 cm to 6 cm deep in a square meter is approximately 20 liters, so that the defecation rate in kilograms per square meter per annum divided by 20 gives us an indication of the number of times the preferred feeding layer has been passed through a worm in a year. These values range from nearly 12 times at zone 1 to about once at zone 4 (Brinkhurst, 1970).

ACKNOWLEDGMENTS

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Bacteriological Evaluation of Flounder (*Hippoglossoides platessoides*) Frozen at Sea and Thawed by Water Immersion[1]

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Hayward, M. J., W. A. MacCallum, and D. H. Shaw. 1970. Bacteriological evaluation of flounder (*Hippoglossoides platessoides*) frozen at sea and thawed by water immersion. J. Fish. Res. Bd. Canada 27: 1983–1986.

Transfer of bacteria from frozen flounder (*Hippoglossoides platessoides*) to the water in which they are immersion-thawed was evaluated in a laboratory-scale test. After 6 hr of batch thawing, the water quality was still acceptable. Transfer of bacteria from the thawing water to fillets processed from these fish was minimal. No correlation was evident between the time the thawing water was in use and the bacterial count on the fillets. It was also determined that the frozen fish on removal from storage had low bacteria counts. In no instance were faecal coliforms observed.

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INTRODUCTION

With the introduction of the immersion water thawer (Anon., 1967) to defrost blocks of fish frozen at sea, a laboratory-scale experiment by batch-thawing was planned to determine the numbers of aerobic bacteria, coliforms, and faecal coliforms present on frozen whole, slash-gutted flounder (*Hippoglossoides platessoides*) on removal from cold storage. It was also of interest to know to what extent these bacteria were transferred to the thawing medium during a 6-hr thawing period. New batches of fish were added (at 1-hr intervals) after removal of the fish thawed during the previous hour. Bacterial counts on skinned fillets were determined after preparation from fish thawed in water with different levels of contamination. Counts on skinned fillets prepared from fish frozen at sea are low (Hayward and MacCallum, 1969). To enhance the carry-over of bacteria from thawing water to fillets, the washing operation used in commercial practice was omitted. If washing had been introduced at this point, a reduction of surface load ranging from 80 to 97% could be expected (Castell, MS, 1953; Department of Fisheries of Canada, 1959; Dyer, 1947; MacCallum et al., 1963).

METHODS AND MATERIALS

The fish were 100-lb (45.5 kg) blocks of flounder that, immediately after catching, had been slash-gutted, washed, and frozen at sea in vertical plate freezers. On landing, the frozen blocks were wrapped in polyethylene, and held in cold storage at −34 C.

For the experimental work, three blocks were removed from cold storage and held at −3.9 C for 24 hr, after which it was possible to separate the still-frozen fish into two approximately 50-lb batches per block. From one of these batches from each block, two 20-lb (9.08-kg) lots were taken to determine the initial state, representing the sanitary state of the fish after removal from

[1]The 20th in a series of papers on quality of Newfoundland fish.

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storage. Two 20-lb lots from the duplicate 50-lb batches were used for the tests of immersion thawing. This thawing was carried out in a plastic tank filled with water, temperature being maintained at 21 C by hot water circulating through a copper heat exchanger.

To determine the initial bacterial count on the frozen fish, the two 20-lb lots were each placed in a container with 60 lb of fresh potable water (a ratio of fish to water of 1:3) at 7.2 C, and washed for 10 min with manual agitation. This time was considered optimum to defrost the ice and loosen the slime from the surfaces and gut cavity of the fish, and to establish an equilibrium between the bacteria present on the fish and those transferred to the water. Water samples were taken for bacterial counts both before and after the fish were immersed.

To measure the accumulation of bacteria from the thawing fish, the duplicate 20-lb lots from each of the three blocks were added consecutively to the same water, at 1-hr intervals. A 1:3 rat io offish to water was maintained with each lot thawed; thus under the most adverse conditions 120 lb of fish were thawed in the same 60 lb of water (a 2:1 ratio of fish to water). The water temperature was 21 C. Water samples were taken for bacteria counts both before and after the fish were immersed.

Further to this, the carry-over of bacteria from the thawing medium to the processed fish was determined by sampling the fillets prepared, under favourable sanitary conditions, immediately after thawing.

Bacteriological Procedure

Water quality was assessed at each stage by measurement of total plate count (TPC) (expressed as the mean number of organisms/ml), and by coliform and faecal coliform estimation (expressed as organisms/100 ml). Coliform estimation was performed as previously described (Hayward and MacCallum, 1969). Faecal coliforms were estimated by loop transfers from coliform positive tubes of Brillant Green Bile Broth to E.C. medium (Hajna and Perry, 1934) incubated in a water bath at 45.5 ± 0.1 C. Apart from the substitution of Difco plate count agar (23.5 g in 1000 ml distilled water at *p*H 7.0) for Nutrient Agar, the methods used for the determination of total plate count have been described elsewhere (Hayward and MacCallum, 1969).

The fillets prepared from the thawed fish were examined by aseptically transferring a sample (100 g) to a sterile blender jar, mixing with 0.1% peptone water (300 g), and homogenizing at high speed for 1 min. An aliquot (40 g) of the homogenate was placed in 0.1% peptone water (60 ml), giving a 1:10 dilution. Serial decimal dilutions were used in the examination for total plate count and coliforms (Department of Fisheries and Forestry of Canada, 1968, p. 7). Tests for faecal coliforms were performed as described for the water samples. Using the methods described above, the results were expressed as the means of organisms/g, and organisms/100 g, respectively.

The complete experiment was repeated four times, each time with three blocks of fish. Results are expressed as the averages of the five experiments, followed by the standard deviation from the mean.

RESULTS AND DISCUSSION

In measuring the initial contamination on the fish, the coliform-free water used had a low total plate count (182 ± 47 organisms/ml, $n = 5$), considered to be insignificant. The average total plate count in the water after the 10-min washing period was 91,766 ± 7229 organisms/ml ($n = 30$). Three of the 15 blocks used were free of coliforms, and the remainder showed counts ranging from 1.8 to 13.3 organisms/100 ml, the average of the coliforms in the 15 blocks being 4.4 ± 1.0 ($n = 30$). In no instance were any faecal coliforms observed.

Each of the five thawing operations started with water that was coliform-free and low in total plate count.

As thawing progressed, coliforms appeared in the water, and these, with the total count, increased with time (Table 1), though even at 6 hr the water

TABLE 1. Bacterial counts (tabulated as the mean followed by the standard deviation).

Time water in use (*hr*)	Thawing water		Skinned fillets		
	TPC/ml ($n = 5$)	Coliforms/100 ml ($n = 5$)	n	TPC/g	Coliforms/100 g
0	182±47	0			
1	89,600±22,950	11.6±7.9	10	12,550±4,013	10.8±5.5
2	180,000±24,240	15.3±7.3	10	13,400±2,650	8.4±4.7
3	240,000±42,870	45.6±17.7	8	12,875±4,320	0.4±0.5
4	252,000±56,880	84.4±41.6	10	14,200±2,995	7.0±3.3
5	314,000±35,900	170.2±82.3	6	22,332±6,130	0.9±0.9
6	395,000±90,000	169.0±82.7	8	17,500±2,810	1.5±0.2

could not be considered to be seriously contaminated either with coliforms or aerobic bacteria. There was no contamination at any stage with faecal coliforms. The logarithmic rate of increase of the aerobic bacteria was greatest during the initial 2-hr period, after which the rate settled down to a slow but steady increase up to the 6-hr period; coliforms, however, showed a more constant increase, but took longer to reach a steady state (Fig. 1).

Although the water after 6 hr use was bacteriologically in good condition, it was considered at this stage to be unsatisfactory because of the amount of slime and dirt that had been removed from the fish. The water was slimy to the touch and visibly dirty. The cause of such slime and dirt build-up is the high ratio of fish to water (2:1) over the 6-hr thawing period. In a continuous thawing operation using commercial equipment (Anon., 1967; MacCallum, 1969) it could require 48 hr to reach this ratio. The maximum period during which the commercial thawer has been operated using a single filling of water is 12 hr (Hayward and MacCallum, 1969; MacCallum, 1969); thus in the present laboratory-scale tests the concentration of slime and dirt in the water probably exceeded to a considerable degree that encountered in commercial thawing.

The sanitary state of the fillets prepared from the thawed fish was good throughout (highest total plate count 40,000 organisms/g), and was independent of the time the water had been used,[2] and apparently not affected by continued use of the same filleting boards. Only 14 of the 52 samples were positive for coliforms, these ranging from 3.1 to 36 organisms/100 g. None of these 14 samples were positive for faecal coliforms.

It is confirmed from these results that the sanitary state of the flounder, on removal from frozen storage, is most acceptable, and indicates that there is little contamination during handling and freezing at sea and during frozen storage aboard the vessel. The bacteria on the frozen fish are transferred to the thawing water, the quality of this water changing due to bacterial growth, addition of bacteria from fresh batches of fish, and contamination of the water by both slime and dirt. Under the favourable conditions used in this work,

[2]A standard statistical test showed that the regression coefficient did not differ significantly from zero.

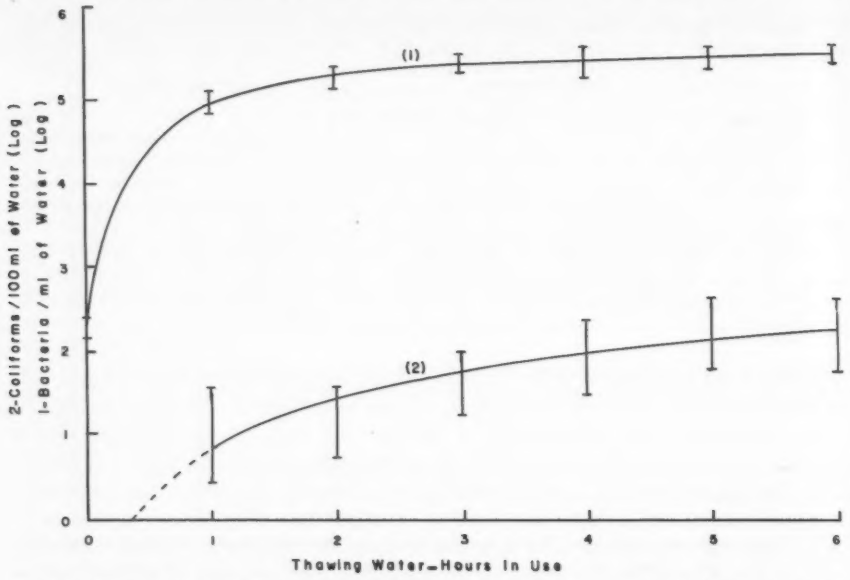

FIG. 1. Increase in total plate count and coliforms with continuous use of thawing water in defrosting frozen-at-sea flounder. Both curves were drawn using the best fit to the mean values.

the latter would appear to be the limiting factor, though at any period up to 6 hr the quality of the thawing water does not affect the fillets prepared from the thawed fish.

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Meristic and Lactate Dehydrogenase Genotype Differences in Stream Populations of Rainbow Trout Below and Above a Waterfall

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Rainbow trout (*Salmo gairdneri*) from above a waterfall on Kokanee Creek had significantly higher average number of parr marks and scale rows but lower number of vertebrae compared with those living below the falls. Three phenotypic forms of liver lactate dehydrogenase (LDH) were recognized in Kokanee Creek rainbow trout (two single-banded homozygous forms CC and C′C′ and a five-banded heterozygous form CC′), apparently under the control of a two allele system. Homozygote CC was predominant in trout from the above falls population and homozygote C′C′ was predominant in those from below the falls. Possible origins of meristic and LDH differences between above- and below-falls populations are discussed and their significance to differences in behaviour, particularly migratory, are considered.

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INTRODUCTION

THE PROBLEM OF explaining patterns and mechanisms in lakeward migration and residence of trout populations from streams below and above falls forming barriers impassible to upstream movement of fish was considered briefly by Northcote (1969). Data given therein for Kokanee Creek, a tributary to Kootenay Lake, B.C., suggested that there were marked differences in migratory behaviour of young between "headwater" (above falls) and "below falls" populations — the former showing little evidence of downstream movement, the latter exhibiting a lakeward migratory pattern characteristic of inlet stream spawners. Differences in migratory behaviour between the two populations did not seem to be controlled by obvious environmental factors (e.g. temperature), and it was suggested that innate differences in current response might be expected in small headwater populations being subjected to strong selective pressure for maintenance of position in streams. Such is apparently the case

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for a population of brown trout (*Salmo trutta*) living above falls on the Swedish river Verkeån (Svärdson and Anheden, 1963; Svärdson and Nilsson, 1964).

In addition to differences in migratory behaviour between headwater and below falls populations in Kokanee Creek, there were also external differences in colouration and appearance. Indeed, headwater forms in nearby streams have been recognized as a subspecies (*whitehousei*) of *Salmo gairdneri* by Dymond (1932) on such features, in combination with other characters, especially lateral line scale rows. Therefore it seemed pertinent to examine headwater and below falls rainbow trout of Kokanee Creek in more detail. The populations were compared by two diverse approaches: (*a*) meristically by counting parr marks, scale rows, vertebrae, and gill rakers; and (*b*) biochemically by using electrophoretically definable proteins as genetic markers.

The biochemical system chosen was lactate dehydrogenase (LDH) because this enzyme has been investigated on 30 species of teleost fishes (Markert and Faulhaber, 1965), and because the tetrameric structure (Appella and Markert, 1961; Markert, 1962) and the molecular bases (Markert, 1968) for isozyme forms are well understood. In salmonid species, some or all of the LDH systems have been described by several investigators (Goldberg, 1965, 1966; Morrison and Wright, 1966; Hochachka, 1966; Ohno et al., 1968; Klose et al., 1968; Bouck and Ball, 1968; Massaro and Markert, 1968; Williscroft and Tsuyuki, 1970), and different systems of nomenclature adopted. In a previous publication (Williscroft and Tsuyuki, 1970) describing the LDH systems of rainbow trout, the polymorphic liver subunits were designated C and C′ in accordance with the convention established by Markert (1962) for the A (skeletal muscle) and B (heart muscle) subunits and with that by Odense et al. (1969) for the C (liver tissue), D (eye tissue), and E (other nonspecific tissue) subunits. The designation C has been used for LDH from gonadal tissue (Zinkham et al., 1963) as well as that from retinal tissue (Morrison and Wright, 1966) and the need for preempting its usage for testicular tissue (Massaro and Markert, 1968) is not universally adopted.

Geographic frequency differences in LDH alleles were observed in *Clupea harengus harengus* (Odense et al., 1966), *Merluccius productus* (Utter and Hodgins, 1969), and *Oncorhynchus nerka* (Hodgins et al., 1969). Recently polymorphism in the LDH from the liver of rainbow trout, and hence of immediate value to this study, was reported for the first time (Williscroft and Tsuyuki, 1970).

GEOLOGICAL AND RECENT HISTORY OF KOKANEE CREEK

Virtually the whole drainage basin of Kokanee Creek is underlaid by a lower cretaceous formation (Nelson plutonic rocks) formed mainly of porphyritic granite (Little, 1960). The creek enters the West Arm of Kootenay Lake. This large lake, during glacial retreat in late Pleistocene, was dammed by a terminal moraine near the big bend of the Kootenay River in the United States, and drained south over the moraine (Schofield, 1946). The lake level

was much higher than at present as evidenced by old deltas some hundreds of feet above those of today at mouths of tributary streams (Little, 1960; W. H. Mathews, personal communication) so that the present falls would not have been a barrier to upstream migration of fish if present in the lake then.

The Kokanee Creek system has been subject to a long and confused history of fish introductions through activities of several hatcheries operating in the region. It is probable that rainbow trout existed above the falls prior to introductions by man, since they do so in other nearby small headwater lakes and streams tributary to Kootenay Lake, such as in Six Mile Lake and in Duhamel Creek, 8 km southwest of Kokanee Creek. The first recorded introduction of rainbow trout to Kokanee Creek was made in 1914 when several thousand young Kootenay Lake rainbow (Lardeau River–Gerrard stock) were apparently planted in Kokanee Lake at the head of the system. However, these may not have survived, at least in the lake, as Weldon Reid, Superintendent of Nelson Hatchery recorded in his report of 1930 (Department of Fisheries, 1932) that "cutthroat introduced into Kokanee and Kaslo lakes have done well and specimens up to two pounds in weight have been reported from the former. These lakes ... were regarded as barren before they were stocked from this hatchery." Since 1914 eyed rainbow trout eggs of unrecorded origin were planted in the creek in 1931, 1942, and 1951; rainbow fingerlings were also stocked in the creek in 1950. Although the location of these plantings was not recorded, most, if not all, were probably made below the falls. Origin of the stocks also was not recorded.

Since the first introduction, which was noted above, of Yellowstone cutthroat trout (*Salmo clarki lewisi*) into the system, this species has been planted in Kokanee Lake in 1931, 1933, 1938, 1939, 1941, 1952, and 1968. It also occurs in Gibson Lake, about 4 km downstream from Kokanee Lake and was stocked there in 1968. Cutthroat trout may be found in Kokanee Creek for at least 5 km below Gibson Lake (Conservation Officier R. A. Rutherglen, personal communication).

METHODS

Rainbow trout were collected from below the falls by seining in the lowermost 0.7 km of the creek. Twenty-six specimens (90–200 mm fork length; largely yearlings) were taken there on September 9, 1969, 25 on September 12 (46–65 mm; young of the year), and 31 on November 14 (45–57 mm; young of the year). An additional 50 yearling or older fish were caught in the below-falls section of the creek on April 23–25, 1970, and used only for parr mark counts. On September 4, 1969, 38 rainbow trout (111–170 mm; yearlings or older) were angled from above the falls in a section 6.5–12.5 km from Kootenay Lake. Several cutthroat trout also were caught in the upper reaches of this section. Another 26 rainbow trout, but no cutthroat, were seined and angled in an above-falls section of the creek 5.5–6.5 km from the lake on September 9, 1969.

Most fish were frozen shortly after capture. Later they were thawed, measured, weighed, parr marks counted, and X-rayed before being preserved for 48 hr in a 10% formalin solution and then transferred to a 40% isopropyl alcohol solution.

All counts were made from the right side of the specimens. Parr marks were counted if they appeared to extend as a single mark above and below the lateral line, although slight "breaks" were occasionally evident in some marks where intersected by the lateral line. For counts of scale rows above the lateral line each specimen was blotted dry of isopropyl alcohol, pressed against

a blue ink stamp pad, reblotted, and then scale rows were counted under a low-power binocular dissecting microscope, using a fine hair across one ocular. This procedure facilitated scale counting by making the rows stand out clearly, even on small fish. Total number of vertebrae (not including the hypural plate) was counted from X-ray photographic negative plates using a dissecting microscope with suitable back lighting. The right anterior gill arch was removed from each specimen, washed, dried briefly under a fine air jet, and rakers counted under a dissecting microscope.

Scale row counts for young-of-the-year fish were not included in the analysis. Although all appeared to have completed scale formation (many showed 3–5 circuli), counts averaged lower than those from the other below-falls collection of yearling and older fish and were not included as they may have biased the total sample in favour of showing a difference when compared with the above-falls collection (Fig. 1). Differences between average counts of each meristic character were compared for significance by *t*-tests, using appropriate adjustment for unequal sample size where necessary.

For electrophoresis, liver samples were excised from partially thawed fish. Liver extracts were prepared by homogenizing approximately 0.3 g of tissue with 4 volumes of chilled sodium phosphate buffer, ionic strength 0.05, *p*H 7.55. After centrifugation the supernatant was applied directly to the starch gel.

The starch gel was prepared as a 13% solution in a buffer (Clayton and Gee, 1969) consisting of 0.002 M citric acid brought to *p*H 8.0 with tris (hydroxymethyl) amino methane (Tris). The electrode buffer was made of 0.04 M citric acid brought to the same *p*H with Tris. Electrophoresis was carried out at 4 C at 200 v for 2½ hr using an apparatus described previously (Tsuyuki et al., 1968).

The histochemical solution for visualizing the lactate dehydrogenase consisted of the following in 200 ml final volume: 40 ml of 0.1 M Tris brought to *p*H 8.0 with HCl, 1.92 g lithium lactate, 60.0 mg of nicotinamide adenine dinucleotide, 3.0 mg of phenazine methosulphate, and 40 mg of nitroblue tetrazolium. The gels were placed in suitable washing trays, incubated with this mixture for 30 min at room temperature, and then washed with water. The zymogram was photographed immediately and also reexamined after 24 hr for the development of any minor components.

RESULTS

Significant differences in several of the meristic characters examined were evident in below- and above-falls populations of rainbow trout (Fig. 1). Compared with those from below falls, the above-falls fish had higher average number of parr marks (12.36 cf. to 10.57), higher average number of oblique scale rows above the lateral line (145.98 cf. to 135.65), and lower average number of vertebrae (63.75 cf. to 64.44). Although the average number of gill rakers in above-falls fish were lower than those below falls in all collections, differences were significant ($P < 0.05$) only in some comparisons (September 9, November 14, below falls cf. to September 4, 9 above falls; September 9 below falls cf. to September 4 above falls). As it could not be definitely determined that full raker numbers were formed in small young of the year, all of this age class were eliminated from the final comparison made in Fig. 1 where the calculated t value (1.88) was below the $P = 0.05$ level (1.99).

The lactate dehydrogenase in the liver of rainbow trout from Kokanee Creek existed in three phenotypic forms (Fig. 2) consisting of two single banded homozygous forms CC and C′C′, and a five-banded heterozygous form CC′. The autotetramer involving the more negatively charged allelic subunit has been designated C'_4 and that involving the less negatively charged component

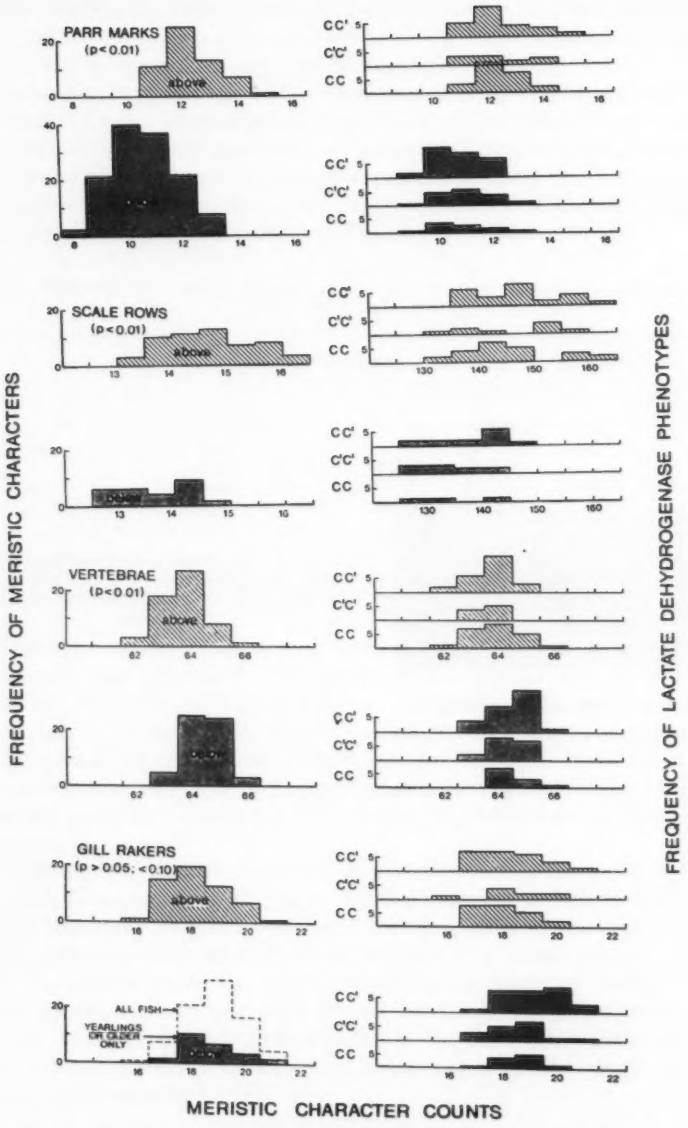

FIG. 1. Frequency distributions of four meristic characters in populations of rainbow trout below and above falls on Kokanee Creek; *P* values in parentheses give significance level of differences between populations. Histograms at right show distribution of CC, C'C', and CC' type liver LDH patterns for meristic characters in each population.

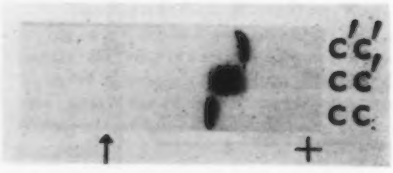

FIG. 2. Starch-gel zymogram showing rainbow trout phenotypes C'C', CC', and CC as expressed in liver LDH. Arrow indicates the origin.

has been labelled C_4 (Williscroft and Tsuyuki, 1970). The five-banded phenotype consists of the two autotetramers and the three heterotetramers, C'_3C, C'_2C_2, $C'C_3$ in approximately binomial proportions.

Trout homozygous for CC predominated in the headwater population of Kokanee Creek. Phenotypic distributions of collections from below and above falls were in good agreement with Hardy–Weinberg expectations (Table 1) indicating that in liver this isozyme protein is under the control of a two allele system. Confidence limits for gene frequencies of the two populations do not overlap and q^C is, therefore, significantly different.

TABLE 1. Distribution of LDH phenotypes in rainbow trout from above and below falls on Kokanee Creek, B.C.[a]

Location	No.		Phenotypes CC	C'C'	CC'	Gene frequency q^C	$q^{C'}$	χ^2
Below falls[b]	57	Obs.	11	17	29	.447	.553	.048
		Exp.	11.389	17.431	28.180			
Above falls[c]	64	Obs.	27	10	27	.633	.367	.544
		Exp.	25.644	8.620	29.736			

[a]95% confidence limits q^C below = .447 (.357–.537); q^C above = .633 (.549–.717).
[b]From collections made below the falls on September 9 and November 14, 1969.
[c]From collections made above the falls on September 4 and 9, 1969.

The four meristic characters (parr marks, scale rows, vertebrae, and gill rakers) were compared with LDH phenotypes in Fig. 1. Within each population, no striking differences in the frequencies of the meristic characters were observed between individuals possessing the two homozygous or the heterozygous LDH phenotypes. However, between populations, the differences in the distributions of the 3 LDH phenotypes with respect to each of the meristic characters generally paralleled the frequency differences in these meristic characters.

Eighteen Yellowstone cutthroat were recognized on the basis of diagnostic characters (Carl et al., 1967) from the September 4 collection further above the falls than that on the September 9 location. This species has a liver LDH isozyme electrophoretically identical to the C'_4 autotetramer of rainbow trout. For convenience, the same subunit designations are used for the two species, although it is by no means certain that the polypeptides are sequentially identical. Phenotype C'C' predominated in the liver LDH isozymes of these cutthroat, the observed distribution being 2CC, 15C'C', and 1CC' in the 18 specimens examined.

DISCUSSION

Meristic characters reported herein for the population of rainbow trout living above falls in Kokanee Creek are similar to those described originally for "mountain" Kamloops trout in the Kootenay area by Dymond (1932),

Mottley (1936, 1937), Carl and Clemens (1948) and for "dwarf forms" from cold headwaters by Carl et al. (1967). In general they are characterized by high numbers of parr marks and scale rows but low vertebral and gill-raker counts (Table 2). On the other hand trout from below Kokanee Creek falls

TABLE 2. Comparison of some meristic counts (range, mean) in different forms of nonanadromous rainbo described in British Columbia.

Source	Location	Form	Parr marks	Scale rows	Vertebrae	Gill
Kokanee Cr.	Above falls	Headwater stream resident	11–15 (12.36)	131–163 (145.98)	62–66 (63.75)	16-
	Below falls	Lower stream resident or lake migratory	8–13 (10.57)	125–147 (135.65)	63–66 (64.44)	17-
Kootenay area (Mottley, 1934, 1936, 1937)	Six Mile Lake	Headwater lake resident	– (–)	– (–)	62–66 (63.57)	- (-
	Kootenay Lake	Lake migratory	– (–)	130–160[a] (144.84)	64[b] (64.00)	- (-
			– (–)	125–146[c] (135.61)	63–66[c] (64.39)	- (-
British Columbia (Dymond, 1932)	Six Mile, Bear, Fish lakes; W Kootenay area	Headwater lake resident	– (about 13)	140–164 (about 150)	– (–)	17-
	Lower elevation inland lakes	Lake migratory	8–13 (usually 9 or 10)	130–160[d] (about 145)	– (–)	18- (-
British Columbia (Carl & Clemens, 1948)	Six Mile, Cottonwood, Bear, Fish lakes; W Kootenay area	Headwater lake resident	– (about 13)	150–155 (–)	– (–)	- (-
	Lower elevation inland lakes	Lake migratory	– (usually 9 or 10)	135–150 (–)	– (–)	- (-
British Columbia (Carl et al., 1967)	High elevation lakes and streams	Headwater lake and stream resident	– (–)	"Highest for fish of cold headwater areas"	– (–)	- (-
	Lower elevation inland lakes	Lake migratory	9–13 (–)	– (about 145)	– (–)	- (-

[a]216 adult rainbow trout from Kootenay Lake and Lardeau River spawning area (Gerrard).
[b]12 adult Lardeau River (Gerrard) spawners.
[c]150 offspring from [b], reared at Nelson Hatchery.
[d]"Ninety percent, however, have from 135 to 153 scale rows."

(be they stream resident or juvenile lake migratory forms) have relatively low numbers of parr marks and scale rows but higher average vertebral and gill-raker counts, similar to rainbow found in Kootenay Lake itself or in other inland British Columbia lakes at lower elevations (Table 2). At least some of these meristic characters are subject to environmental modification (for example by temperature) as clearly shown by experiments of Mottley (1934, 1937). However, marked differences in environmental conditions, especially temperature, are not evident between above- and below-falls sections of Kokanee Creek (Northcote, 1969). Furthermore, some of the meristic differences, for example in vertebral number, are contradictory to those that would have been predicted on the basis of temperature-induced changes. Because of probable differences in spawning time between below- and above-falls populations (the former earlier than the latter), developing eggs may be subject to different thermal regimes and hence show meristic differences affected by temperature. However, the ultimate cause may be an innate difference in spawning period.

The observed frequency of the C allele in populations above the falls represents a minimum value as the elimination of the Yellowstone cutthroat trait from the gene pool would have the effect of lowering the number of the C′ allele that predominates in this species. Only those specimens that could definitely be recognized as Yellowstone cutthroat were excluded from the above-falls collections for LDH analyses. It is possible, therefore, that the above-falls rainbow trout population may have been at one time entirely the rare homozygous CC form. However, in the below-falls collections, the observed frequency of the C allele is probably a maximum value. Random escapement over the falls of trout with high C allele frequency would have the effect of increasing the number of C alleles relative to the C′ alleles. Furthermore, Yellowstone cutthroat were not recognized in the below-falls collection, nor were hybrid individuals apparent, thus minimizing the contribution of the C′ allele from this source. The possibility that the below-falls rainbow trout populations at one time could have been exclusively the common homozygous C′C′ form is not eliminated.

Although no obvious meristic differences were observed between homozygous phenotypes CC and C′C′, it is possible that some might be found if larger samples were used. A number of other protein systems could also be investigated, and if polymorphic, may show phenotypically linked morphological differences. One other protein system examined, the blood hemoglobins, was identical in the two populations. It is possible that the phenotypic differences in liver LDH could affect behavioural patterns such as the migratory differences observed by Northcote (1969) in the two populations of rainbow trout from this same stream. The directional response to water current of young from known above- and below-falls parents is being tested and some differences are evident. Furthermore the current responses and endurance of young from pure CC and C′C′ LDH selected strains will be compared to investigate the possibility of different LDH phenotypes being reflected in behavioural and physiological characteristics.

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Postmortem Glycolytic and Other Biochemical Changes in White Muscle of White Sucker (*Catostomus commersoni*) and Northern Pike (*Esox lucius*) at 0 C

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Postmortem biochemical changes in the white muscle of white sucker and of northern pike were, in general, similar to those observed in other species as reported previously by other investigators. However, glycogen content of pike was found to remain relatively high even after 7 days of storage in ice. This is in contrast to the findings with several other species, including white sucker, where the muscle glycogen is practically completely degraded in 3–4 days. Higher glycogen content in the posterior portion of pike muscle as compared with that in the anterior portion may partly explain the apparent high glycogen content in the muscle of this fish after several days of storage.

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INTRODUCTION

SEVERAL STUDIES have been reported in literature regarding the glycolytic and related biochemical changes that occur in the early postmortem period in the white muscle of marine fishes, but similar studies with freshwater species are few in number. The marine fish so far studied include cod (Fraser et al., 1961, 1967), haddock (Macpherson, 1932), sockeye salmon and starry flounder (Tomlinson et al., 1961), frigate mackerel (Amano et al., 1953), red snapper (Noguchi and Yamamoto, 1955), plaice (Fijii et al., 1966), halibut (Spinelli, 1967), and three Indian fishes — mullet, Bombay duck, and grouper (Nazir and Magar, 1963). The freshwater fishes include only rainbow trout (Tomlinson et al., 1961; Kuusi and Aalto, 1968) and carp (Noguchi and Yamamoto, 1955; Tomiyama et al., 1966). Good reviews of these studies have been published (Tomlinson and Geiger, 1962; Partmann, 1965; Tarr, 1966). The only reference related to pike (*Esox lucius*) is by Kuusi and Aalto (1968), who described some incomplete experiments in which they measured labile phosphorus (labile-P), creatine phosphorus (Cr-P), inorganic phosphorus, and adenylic radicals in pike muscle during storage at 4 C for 4 days.

The general pattern of the postmortem biochemical changes appears to be as follows: glycogen content decreases during postmortem period and glycolysis is practically complete by the time the fish is in full rigor; simultaneously, there is an increase in lactic acid although a stoichiometric relation between glycogen lost and lactic acid formed is not always observed. Muscle *p*H, normally around 7.0 at the time of death, decreases until a value of 6.2–6.6 is reached. Cr-P and easily hydrolyzable phosphate (labile-P), of adenosine triphosphate (ATP) and adenosine diphosphate (ADP), decrease during storage

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and are practically completely lost by the time the fish is in rigor. Cr-P seems to be lost earlier than the labile-P. The rates at which these changes take place depend on the temperature at which the fish is stored in the postmortem period; the higher the temperature the more rapid the biochemical changes.

The present study was undertaken to investigate the postmortem changes in glycogen, lactic acid, Cr-P, labile-P, and *p*H in the white muscle of two freshwater fishes, white sucker (*Catostomus commersoni*) and northern pike (*Esox lucius*). These are two of the more common fishes found in the rivers and lakes of central Canada.

MATERIALS AND METHODS

Fish, weighing 1–3 lb each, were captured by gillnets in the Red River near Selkirk, Man. throughout the year. They were kept alive in an aquarium for several days. During captivity, white suckers were fed with fish pellets, and northern pike with live minnows. As needed, the fish were removed from the aquarium with a hand net and killed immediately by a blow on the head. They were then beheaded, gutted, washed with cold tap water, and stored in cracked ice. At regular intervals, a complete transverse cut was made to remove a steak of about 1½ inches in thickness from which the lateral dorsal muscle was removed for chemical determinations. Only the white muscle was used, the red muscle being carefully avoided. Successive samples were obtained starting from the anterior end. Extreme tail muscle was not used. Chemical determinations were done with duplicate samples of the muscle.

Glycogen was isolated by potassium hydroxide digestion and precipitation with ethanol according to Good et al. (1933) and measured by the phenol–sulphuric acid method of Montgomery (1957). Lactic acid content was determined on a trichloroacetic acid extract of the muscle by the method of Barker and Summerson (1941). Muscle *p*H was measured with a glass electrode in a homogenate of 1 g muscle in 7 ml 0.005 M sodium iodoacetate (Marsh, 1952). Cr-P was measured as molybdate-labile phosphate from the trichloroacetic acid extract by the method of Fiske and Subbarow (1929).

To determine the labile-P of nucleotides (mainly from ATP and ADP), the nucleotides in the trichloroacetic acid extract were first adsorbed on activated charcoal (Norit A) and, after washing the charcoal with distilled water, were hydrolyzed for 10 min by treating the charcoal with 1 N HCl in a boiling water bath (Crane and Lipmann, 1953). Orthophosphate released from charcoal in the solution was then measured by the method of Fiske and Subbarow (1925). Hexose phosphates and Cr-P are not adsorbed on charcoal. Although adenosine monophosphate and inosine monophosphate are adsorbed on charcoal, no orthophosphate is released from them in solution in the above procedure. Rigor mortis was observed by holding the fish by the head end sideways and horizontally and noting the rigidity of the body.

RESULTS

All the fish were found to be in rigor generally 2 or 3 days after death and the rigor was completely resolved in 2 more days.

Sucker — The biochemical changes observed in the muscle of 14 white suckers during 7 days of storage are summarized in Table 1. Glycogen, Cr-P, and labile-P decreased continuously until practically completely degraded after 3 days. Lactic acid decreased during storage until a maximum was reached on the 3rd or 4th day after which it showed a slight decrease. The *p*H was found to decrease from about 7.0 to 6.5 during 7 days of storage.

TABLE 1. Biochemical changes in the muscle of white sucker stored in ice.

s postmortem		0	1	2	3	4	7
gen	Mean ± SE	369±56	142±31	49±21	10±3	6±3	2±0.5
/100 g)	Range[a]	130–824	2–333	1–261	1–37	1–30	1–4
	No. fish	14	14	14	14	10	7
c acid	Mean ± SE	124±18	222±22	318±34	327±20	326±31	298±28
/100 g)	Range	51–172	140–281	161–394	254–400	252–411	184–400
	No. fish	6	6	6	6	5	6
	Mean ± SE	7.08±0.02	6.85±0.04	6.67±0.04	6.61±0.05	6.62±0.07	6.56±0.06
	Range	6.95–7.20	6.60–7.15	6.50–7.10	6.35–7.00	6.35–7.00	6.40–6.80
	No. fish	14	14	14	14	11	7
(μg/g)	Mean ± SE	114±7	30±3	14±4	6±2	11±3	8±3
	Range	70–150	16–44	0–38	0–16	0–20	3–14
	No. fish	10	10	10	10	6	3
le-P (μg/g)	Mean ± SE	226±22	151±22	38±11	18±3	14±1	17±1
	Range	113–349	29–256	16–141	6–46	13–17	16–18
	No. fish	11	11	11	11	8	3

[a]Range — the rank order of specimens did not stay constant from day to day.

Pike — The biochemical changes observed in the muscle of 46 northern pike are summarized in Table 2. The changes observed in lactic acid, Cr-P, labille-P, and *p*H in pike were similar to those observed in white sucker. However, the changes in glycogen of pike muscle during storage were extremely variable. An appreciable amount of glycogen (greater than 50 mg/100 g) remained in the muscle of 26 out of 44 pikes even after 7 days of storage. In two individual pikes out of four, stored for 21 days, glycogen content of as

TABLE 2. Biochemical changes in the muscle of northern pike stored in ice.

s postmortem		0	1	2	3	4	7
ygen	Mean ± SE	280±22	217±50	151±35	155±24	145±32	99±15
g/100 g)	Range[a]	26–662	47–672	12–423	2–470	0–335	0–437
	No. fish	46	14	15	26	11	44
ic acid	Mean ± SE	130±11	162±29	246±33	313±24	363±37	332±13
g/100 g)	Range	36–289	59–325	105–404	129–455	247–472	164–500
	No. fish	26	9	9	15	5	25
	Mean ± SE	7.01±0.03	6.81±0.06	6.62±0.04	6.59±0.03	6.55±0.06	6.55±0.03
	Range	6.65–7.30	6.40–7.10	6.40–6.90	6.35–6.95	6.35–6.95	6.45–6.95
	No. fish	24	13	14	20	9	18
(μg/g)	Mean ± SE	177±24	60±14	32±9	10±2	8±1	5±1
	Range	68–284	3–147	3–99	0–24	0–13	0–7
	No. fish	11	11	11	10	8	5
le-P (μg/g)	Mean ± SE	221±16	156±25	61±16	26±10	16±1	16±1
	Range	34–320	34–289	17–218	9–132	11–19	13–22
	No. fish	19	13	13	12	8	7

[a]Range — the rank order of specimens did not stay constant from day to day.

high as 185 mg/100 g was observed at the end of that period. In two other individuals, the glycogen content at the end of 7 days' storage was found to be slightly higher than that at the time of death. Glycogen degradation during 7 days of storage was practically complete in only 9 out of 44 pikes.

Because of the difference observed in the pattern of change in glycogen content measured along the length of fish muscle on successive sampling days, it was of interest to know whether the concentration of glycogen varied initially along the length of the muscle and whether this variation was different in the two species. Three white suckers and four northern pikes were examined immediately after killing. White muscle was removed from five sites evenly separated along the length of the fish from anterior to posterior end. The glycogen contents found in the muscle at these sites are shown in Table 3.

TABLE 3. Glycogen distribution (mg/100 g) along the length of the fish muscle. Site 1 is the anterior end; site 5 is the posterior end.

Site	Sucker 1	Sucker 2	Sucker 3	Pike 1	Pike 2	Pike 3	Pike 4
1	414	209	253	311	296	404	16
2	360	189	215	370	444	522	13
3	302	145	219	452	477	583	10
4	295	163	265	473	450	569	12
5	228	91	245	552	478	613	14

In two suckers (1 and 2) the glycogen decreased gradually from head to tail; in the third (3) it was rather constant over the whole length of the fish. Three pikes (1, 2, and 3), on the other hand, showed an increase in glycogen from head to tail. The fourth pike (4) had so little muscle glycogen that no meaningful variation could be observed.

DISCUSSION

Postmortem changes in lactic acid, *p*H, Cr-P, and labile-P in the muscle of white sucker and of northern pike are similar to those observed with several marine and two freshwater species mentioned in the introduction (Table 1, 2).

Fish muscle glycogen is degraded rapidly after death. In relaxed cod, kept at 0 C after death, initial glycogen, about 300 mg/100 g was practically completely lost in 4 days (Fraser et al., 1967). Macpherson (1932) found that in unexercised haddock, kept in ice after death, glycogen content of muscle decreased from 530 mg/100 g to zero in 53 hr. Under similar conditions glycogen decreased from 219 mg/100 g to 26 mg/100 g in mullet and from 393 mg/100 g to 95 mg/100 g in grouper after only 20 hr (Nazir and Magar, 1963). In carp, killed immediately after capture and stored at 30 C, the glycogen decreased from about 2300 mg/100 g to about 500 mg/100 g in 12 hr; in mackerel the degradation of about 2500 mg/100 g glycogen was practically complete in about 10 hr at 20 C, and in red snapper from 1000 mg/100 g in the same

period (Noguchi and Yamamoto, 1955). In the present investigation, glycogen content in the muscle of white sucker was reduced similarly from an average of 369 mg/100 g to 10 mg/100 g after 3 days on ice.

In contrast to the above-mentioned findings the average muscle glycogen of pike decreased from 280 mg/100 g to only 155 mg/100 g after 3 days and to 99 mg/100 g after 7 days in ice. Although the degradation of glycogen in the majority of pikes was not complete even after 7 days, all the fish went into rigor within 2–3 days at which time also the degradation of high energy phosphates was almost complete.

It is generally assumed that postmortem glycolysis will continue until the glycogen is completely depleted or until a *p*H of 5.4–5.5 is reached in the muscle, at which point the enzyme systems producing lactic acid are inactivated and some residual glycogen may be found (Partmann, 1965). In the present investigation, however, *p*H of the muscle of northern pike never went below 6.35.

The gradual increase in glycogen in fresh pike muscle from anterior to posterior end (Table 3) was similar to that observed in frigate mackerel (Amano et al., 1953) and in cod (Fraser et al., 1966). Such distribution of glycogen in the muscle might be considered as an explanation of the presence of appreciable amounts of this substance in postrigor periods. However, even when muscle samples were obtained from the posterior end working towards the anterior end of one pike on successive sampling days, glycogen decreased from 430 mg/100 g to only 72 mg/100 g after 7 days at 0 C. High initial concentration of glycogen in the posterior portion of pike muscle, therefore, does not explain completely the presence of residual glycogen in the postrigor period.

Whether the presence of such residual glycogen, postrigor, is due to the inactivation of glycogen phosphorylase (Ono et al., 1957) or other glycolytic enzymes during storage or whether it is due to the inaccessibility or insusceptibility of this portion of glycogen to these enzymes as suggested by Lawrie et al. (1959) remains to be investigated.

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5′-Nucleotidase of Testes of Immature Sockeye Salmon (*Oncorhynchus nerka*)

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GARDNER, L. J. 1970. 5′-Nucleotidase of testes of immature sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 27: 2003–2007.

Fifty percent of the salmon 5′-nucleotidase is in the soluble fraction after high-speed centrifugation. A threefold purification on a Sephadex G-200 column gives a specific activity of 0.5 μmoles UMP hydrolyzed per hour per milligram of protein. The *p*H activity curve gives a single peak with an optimum at *p*H 9.0 $MgCl_2$, $CaCl_2$, and $MnSO_4$ increase the activity whereas Zn acetate, Ni acetate, and $CoSO_4$ inhibit the enzyme. EDTA, KF 2-mercaptoethanol, and dithiothreitol inhibit the nucleotidase activity. It is stable for up to 1 week at 0 C and up to 2 hr at 35 C, but activity decreases to 50% after 15 min at 50 C and no activity is left after 15 min at 60 C. The nucleotidase shows greatest activity towards 5′-nucleotides; 2′(3′) nucleotides and 5′-deoxynucleotides are hydrolyzed less effectively. Ribose-5-phosphate and *p*-nitrophenylphosphate are hydrolyzed, but no activity is exhibited against fructose-1-phosphate and α-glycerophosphate.

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INTRODUCTION

ENZYMES possessing 5′-nucleotidase activity are of widespread occurrence (Heppel, 1961; Bodansky and Schwartz, 1968). Their subcellular localization has been studied (Song and Bodansky, 1967; Song et al., 1967) and they have been used as marker enzymes for plasma membranes in cell fractionation studies (Ray, 1970; Thines-sempoux et al., 1969; Perdue and Sneider, 1970). This paper describes the partial purification of a 5′-nucleotidase from the soluble fraction of sockeye salmon (*Oncorhynchus nerka*) testes and some of its properties.

MATERIALS AND METHODS

Nucleotides and other biochemicals were obtained from Sigma, Schwartz, Calbiochem, or P.L. Biochemicals. Sephadex G-200 was obtained from Pharmacia.

The following assay was used to determine the activity of crude enzyme preparations or where there were high concentrations of orthophosphate. UMP-^{14}C 25 nmoles, 0.005 μCi, tris (hydroxymethyl) amino methane (Tris) buffer *p*H 8.5 2 μmoles, $MgCl_2$ 0.5 μmoles, and enzyme in a volume of 0.05 ml were held 20 min at 25 C before stopping the reaction by heating 30 sec at 100 C. The amounts of uridine-^{14}C and UMP-^{14}C were measured as described previously (Tarr et al., 1969). For purified preparations the standard assay was as follows: UMP, 2 μmoles; $MgCl_2$, 5 μmoles; Tris buffer *p*H 8.5, 20 μmoles; and enzyme in a volume of 0.5 ml. Tubes were held 30 min at 25 C before stopping the reaction with 0.1 ml of 1 M perchloric acid. The volume was made to 1.075 ml with water and inorganic phosphate was determined by the method of Gomori (1942). Protein in G-200 eluates was determined by absorption at 280 mμ, and by the method of Lowry et al. (1951) in crude preparations and in pooled eluates.

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All procedures were carried out at 0–3 C. The crude enzyme was prepared by homogenizing 7–12 g of immature sockeye salmon testes (weight range 5–10 g) that had been stored at −35 C for 4–8 months, in 2 vol of a solution containing 0.2 M sucrose, 0.05 M Tris *p*H 7.5, and 0.0005 M $MgCl_2$ in a Serval Omni-mixer before centrifuging for 10 min at 1000 *g*. The resulting supernatant solution was retained and the residue was washed once with one volume of homogenizing medium and recentrifuged. The supernatant liquids were pooled and centrifuged 20 min at 20,000 *g*. The residue was discarded and the supernatant liquids were centrifuged 1 hr at 100,000 *g* using a no. 65 rotor in a Spinco L2-65B ultracentrifuge. The 100,000 *g* supernatant solution was concentrated to one-fourth its volume using a Diaflo ultrafiltration cell (Amicon Corporation) and a PM-30 membrane under 40 lb nitrogen pressure. Three ml of concentrated enzyme solution were layered on a Sephadex G-200 column (38 × 3 cm) and eluted at about 30 ml/hr with 0.04 Tris *p*H 8.0 collecting 5-ml fractions. The fractions were assayed and the peak tubes (usually fractions 13–14) were pooled (Fig. 1) and used in subsequent experiments.

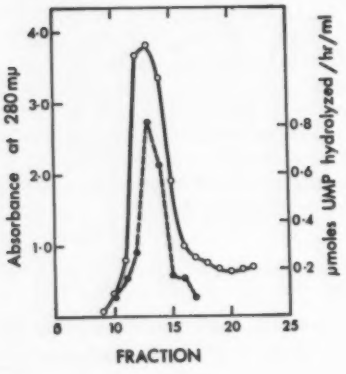

FIG. 1. Elution of salmon testis 5′-nucleotidase from Sephadex G-200: ○ = protein by absorbance at 280 mμ; ● = 5′-nucleotidase by standard assay.

RESULTS

The specific activity of the Diaflo concentrate varied between 0.14 and 0.30 μmoles UMP hydrolyzed per hour per mg of protein with a mean of 0.27. The specific activities of the pooled G-200 eluates varied between 0.23 and 1.6. The highest purification obtained was sevenfold, but it was usually about two- to threefold for a typical preparation. Of the total activity placed on the G-200 column 50–80% was recovered in a single peak of activity. No isoenzymes were separated on the column.

Attempts at further purification using DEAE-cellulose and hydroxyapatite columns or alumina $c\gamma$ resulted in complete loss of activity. When applied to phosphocellulose columns (Yoneda and Bollum, 1965) the enzyme was eluted at very low phosphate concentration (0.002 M) and, although recovery was good (about 80%), there was very little increase in specific activity (SA) (100,000 *g* supernatant solution, SA = 0.27; phosphocellulose eluate, SA = 0.35). Losses in activity also occurred during concentration by Diaflo ultrafilter or "Lypho-gel" (acrylamide gel) and by ammonium sulphate fractionation.

The purified nucleotidase was stable for 1 week at 0 C, and 2 hr at 35 C, but lost 15% of its activity after 1 hr at 40 C, 47% after 15 min at 50 C, and

100% after 15 min at 60 C. Dithiothreitol, 2-mercaptoethanol, and 20% glycerol did not protect the enzyme.

The enzyme showed a zero-order reaction rate for up to 1 hr using 4 mM UMP and 200–400 μg of purified enzyme and for up to 30 min using 0.5 mM UMP-^{14}C and 25–50 μg of crude enzyme per assay.

The *p*H optimum was 9.0 using Tris–HCl buffer; glycine–NaOH buffer gave the same optimum, but the activity was depressed about 15%. The *p*H activity curve of the whole homogenate was not determined.

Table 1 shows the effect of metal ions and various inhibitors. Low concentrations of Zn^{2+} and Co^{2+} (.01 mM) stimulated the enzyme, but higher concentrations effected marked inhibition. Ca^{2+} alone increased the nucleotidase

TABLE 1. Effect of metals and inhibitors.

Compound	Concentration (*mM*)	Inhibition or activation
$CoSO_4$	0.01	+28
$CoSO_4$	5.0	−79
$Zn(Ac)_2$	0.01	+11
$Zn(Ac)_2$	0.05	−50
$MnSO_4$	0.01	+13
$MnSO_4$	1.0	+30
CaCl	10.0	+17
CaCl + MgCl	10.0	+70
MgCl	10.0	+40
$Ni(Ac)_2$	0.01	−31
$Ni(Ac)_2$	0.10	−48
$Ni(Ac)_2$	1.00	−79
NaCl	2.0	+16
NaCl	10.0	+33
KCl	2.0	+ 9
KCl	10.0	+22
EDTA (10 mM $MgCl_2$)	1.0	−15
EDTA (10 mM $MgCl_2$)	10.0	−45
KF	1.0	−100
2-Mercaptoethanol	5.0	−53
Dithiothreitol	5.0	−95

activity whereas there was a greater than additive stimulating effect when Mg^{2+} was present in the same concentration. The acid 5′-nucleotidase of *E. coli* (Neu, 1967) also showed this phenomenon when Ca^{2+} and Co^{2+} were both present.

The 5′nucleotides, 5′-CMP, and 5′-AMP were hydrolyzed most rapidly followed by 5′-UMP and 5′-GMP. The 2′(3′) nucleotides were hydrolyzed about one-third as rapidly whereas deoxynucleotides were comparatively poor substrates. Glucose-6-phosphate, fructose-1-phosphate, sodium pyrophosphate, and α-glycerophosphate were not hydrolyzed.

TABLE 2. Substrate specificity of salmon testis 5′-nucleotidase.[a]

Substrate	Relative activity	Substrate	Relative activity
5′-UMP	100	5′-dUMP	28
5′-CMP	109	5′-dCMP	19
5′-AMP	112	5′-TMP	44
5′-GMP	79	5′-dGMP	25
5′-IMP	61	5′-dAMP	38
2′(3′)-AMP	38	ADP	38
2′(3′)-GMP	34	ATP	13
2′(3′)-UMP	34	R-5-P	29
2′(3′)-CMP	37	*p*-Nitrophenylphosphate	34

[a]The standard assay for purified enzyme was used with substrate concentration = 2 mM. The following compounds were not hydrolyzed: Napyrophosphate, α-glycerophosphate, glucose-1-phosphate, fructose-1-phosphate.

Attempts at determining the subcellular distribution of the nucleotidase showed that 29% of the activity was associated with the nuclear fraction. Part of this activity could be solubilized by treatment with 0.5% sodium deoxycholate. When crude "microsomes" were prepared by the method of Mahler and Cordes (1966) 30% of the nucleotidase activity was associated with this microsomal fraction and 70% was soluble. By treating the 20,000 *g* supernatant liquid (see results for method of preparation) with sodium deoxycholate (final concentration 0.8%) and layering 3 ml over 6 ml of 0.5 M sucrose that had been layered over 4 ml of 1.5 M sucrose in a polyallomer tube and then centrifuging for 3 hr at 110,000 *g* in a SW 40 rotor; 100% of the enzyme activity was found in the soluble top layer. The white insoluble layer, which possibly contained ribosomes (Means et al., 1969), was well separated being between the 0.5 and 1.5 M sucrose layers.

DISCUSSION

The *p*H optimum (9.0) in glycine NaOH buffer and substrate specificities were similar to bull seminal plasma 5′-nucleotidase (Heppel and Hilmoe, 1951) whereas the effect of metal ions was quite different. The activating effect of Mg^{2+} on the seminal plasma enzyme was inhibited by Ca^{2+} whereas Ca^{2+} increased the activity of the salmon testis enzyme with or without added Mg^{2+}.

The relative rates of hydrolysis of 5′-nucleotides and deoxynucleotides of fish muscle 5′-nucleotidase (Tarr et al., 1969) were similar to those of testis nucleotidase. The 2′(3′) mononucleotides were not hydrolyzable by the fish muscle enzyme whereas the testis nucleotidase hydrolyzed them at a faster rate then deoxynucleotides. The average specific activity of the testes enzyme (0.27 μmoles UMP hydrolyzed per mg protein per hr) was considerably lower than that of the muscle enzyme (8.7 μmoles). Dithiothreitol, 2-mercaptoethanol, Zn^{2+}, KF, and EDTA inhibited both enzymes. The testis

nucleotidase in common with fish muscle nucleotidase and many other fish enzymes was unstable even at 0 C and in the presence of protective agents.

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Nutrient Supply and Primary Production in Clear Lake, Eastern Ontario

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SCHINDLER, D. W., AND J. E. NIGHSWANDER. 1970. Nutrient supply and primary production in Clear Lake, eastern Ontario. J. Fish. Res. Bd. Canada 27: 2009–2036.

Clear Lake, a small Canadian Shield lake that has been influenced little by human activity, was found to be more productive than expected from chemical and optical properties and geological surroundings. Production during the ice-free season ranged from 0.10 to 2.57 g C/m² per day and averaged 250 g C/m² per year for the lake as a whole. Other limnological factors studied (phytoplankton standing crop, oxygen, temperature, and nutrient regimes) were typical for an oligotrophic lake.

The high production values are partially explained by the high correction for filtration error, which averaged 3.2 times for all occasions tested.

Atmospheric precipitation is undoubtedly the major source of supply of the nutrients nitrogen and phosphorus to Clear Lake. Calcium and magnesium also appear to be largely carried in by precipitation, although most of the sodium, potassium, and silica entering the lake is weathered from the terrestrial watershed.

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INTRODUCTION

THIS PAPER is the first of a series describing the relation of phytoplankton and zooplankton production to physical, chemical, and geological factors in a variety of types of lake basins within the Canadian Shield. Phytoplankton production in Canadian Shield lakes has received little study until recently, in spite of the fact that most of Canada's lakes lie within this geological formation. Johnson et al. (1969a) measured production in three lakes near Sudbury, Ont. Schindler and Holmgren (1971) have prepared a preliminary report on primary production studies of several lakes in the Fisheries Research Board Experimental Lakes Area near Kenora, Ont. They include a review of all phytoplankton work in Canadian Shield lakes. Schindler (1971) compares phytoplankton production in a variety of Shield lakes. Clear Lake is of particular interest because primary production is extremely high for a lake with low phytoplankton standing crop and nutrient supply, and because the production is not reflected in the chemical regimes that are normally used as indicators of lake metabolism (oxygen, hypolimnetic nutrient accumulation, etc.).

Vollenweider (1968a, p. 75) has pointed out the paucity of information on natural rates of nutrient supply (loading) to water bodies uninfluenced

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by cultural activities. Since the Clear Lake system is a simple one, with no inlet streams from other lakes, a single discrete outlet and little or no groundwater flow, we are able to calculate a nutrient "loading rate" for the lake.

Lake Basin — Clear Lake[2] lies in Sherborne Township of Haliburton County, Ont., at 45°11′N and 78°43′W. The drainage basin is completely underlain by acidic Precambrian granite and gneiss. Rowe (1959) classifies the area as the Algonquin–Pontiac Section of the Great Lakes – St. Lawrence Region. The rugged topography exposes bedrock on the steeper slopes with shallow soils over most of the remainder. Scattered deep pockets of soil with a well-developed B horizon do occur within the drainage basin of the lake (Hett, MS, 1969).

The upland forest cover in the basin is predominantly climax hardwoods, maple, beech, and red oak, with yellow birch and hemlock occupying the better sites. A narrow coniferous band of white and red pine, white cedar, balsam fir, and hemlock occupies the lake shore. Much of the shoreline cover originated after a small fire that occurred 60–80 years ago. The upland stands have remained relatively undisturbed by fire or logging. Hett (MS, 1969) estimates the largest mature hemlocks to be 440 years old. In spite of the poor soil and rough topography the vegetation is more lush than that of Shield regions of lower elevation immediately to the south (southern Haliburton and northern Peterborough counties). This probably reflects the higher annual precipitation in the Clear Lake area.

The surface area (A_o) of Clear Lake (Fig. 1, 2) is 88.4 ha, as determined from measurements made on winter ice with a surveyor's tape, and from an outline taken from aerial photographs. The watershed receives no inflow from other lakes. Runoff either enters the lake directly or collects in small inflow streams, all but one of which dry up completely during the summer. A single small stream drains the lake to the south into nearby Big Hawk Lake.

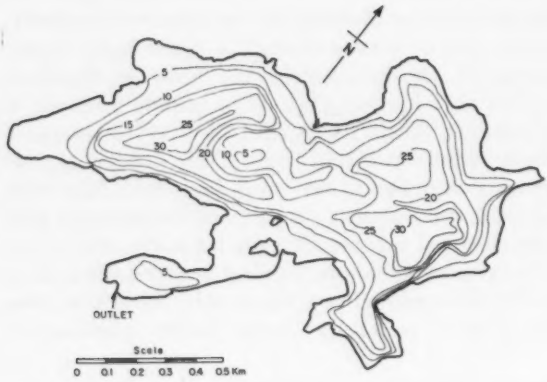

FIG. 1. Morphometric features of Clear Lake. Contours in meters.

[2]The lake should not be confused with other nearby lakes with the same name.

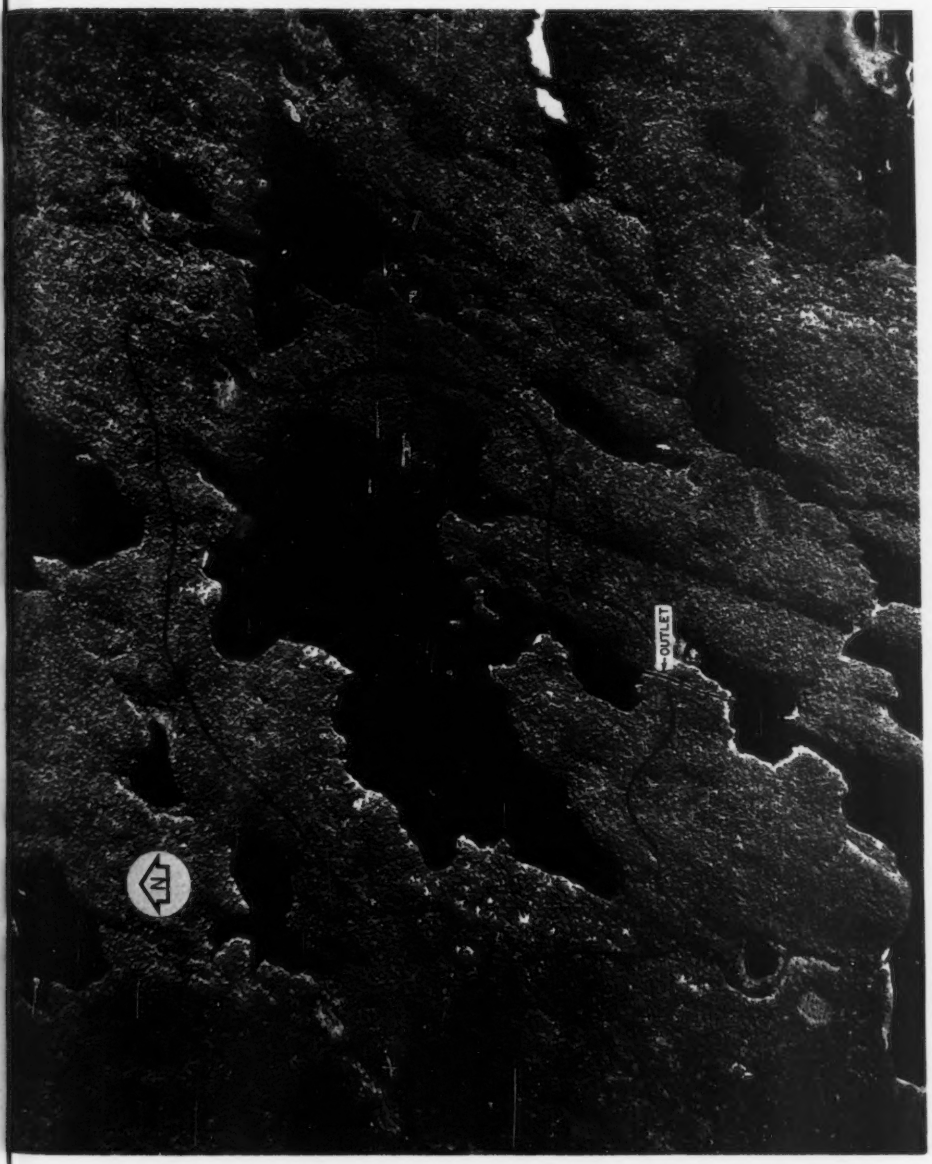

FIG. 2. The Clear Lake drainage system.

The rock bottom and steep shores in nearshore areas are unsuitable for aquatic macrophytes, which are almost completely absent from the lake. Morphometric data follow:

									Large	Small
Depth (*m*)	0	5	10	15	20	25	30	33	island	island
Area (10^4 m^4)	88.4	61.4	47.3	33.8	21.3	10.2	2.6	0	3.6	0.8
Vol (10^5 m^3)	37.6	26.4	20.1	13.7	7.8	3.2	1.6	–	Total =	110.3

Avg depth = 12.5 m; elevation, 369 m

The area of the terrestrial drainage to the lake (A_d) is approximately 125 ha, so that the land area drained is only 1.4 times the area of the lake. This small ratio of drainage area to lake area, the paucity of soil or overburden, and the lack of inflow from other lakes are probably responsible for the extremely dilute waters of Clear Lake, which has lower concentrations of major ions than larger lakes in the immediate area. The insolubility of Shield bedrock is well known (Brunskill and Schindler, 1971).

The lake is well protected from wind, since the rim of the small drainage basin lies 10–75 m above the lake surface, and forest cover adds another 20–30 m of protection. As a result the lake exhibits the shallow thermocline and metalimnetic oxygen maximum that are characteristic of small, clear Shield lakes. Many small, deep Shield lakes including nearby Blackcat (Pot) Lake, exhibit morphometrically induced meromixis due to this high degree of wind protection.

Annual precipitation averages 900 mm/year, of which about 200 mm falls as snow. Evapotranspiration accounts for roughly 530 mm of this. The July mean temperature is 18 C, the January mean temperature is –10 C.

Although there are a small number of summer cabins within the drainage basin these are used infrequently, and the lake is largely uninfluenced by human habitation.

MATERIALS AND METHODS

Phytoplankton production and standing crop — Primary production was measured by the ^{14}C method at weekly intervals during the ice-free period beginning in April 1967, plus several times under ice and snow during the following winter. Water samples from 0, 1, 2, 3, 5, 10, and 20 m were taken with a plexiglass van Dorn bottle from two stations near the maximum depth in the two basins. These samples were placed in 125-ml pyrex bottles, inoculated with 2 μc of ^{14}C as carrier-free Na_2CO_3, and incubated from 10:00 AM to 2:00 PM at the depths taken. Two light bottles were incubated from each depth and station, plus two dark bottles, at 1 m and 20 m.[3] ^{14}C inoculations were made with a Cornwall continuous pipette. Bottles were suspended from a clear glass float. After incubation, a 25-ml aliquot from each bottle was filtered through a 47-mm-millipore HA membrane (0.45-μ pores) using a vacuum equal to 25 cm of mercury. Membranes were stored in plastic petri plates and counted with a Tracerlab automatic gas-flow Geiger counter with a Mono-mol resin window. The activity of ^{14}C stock solutions and the efficiency of geiger counting were checked by liquid scintillation as outlined by Schindler (1966). The publication of Arthur and Rigler (1967), which appeared about midway through the study, caused us to test for bursting of delicate cells during filtration.[4] The exact procedure used is outlined elsewhere (Schindler and Holmgren, 1971). Production was corrected to full-day values by using the ratio

[3]More detailed investigations on a few occasions revealed low ^{14}C uptake in dark bottles and little variation in dark bottle values with depth.

[4]A single set of comparisons between ^{14}C and oxygen methods at seven depths performed in Clear Lake in September 1967 yielded net production values averaging 5% less and gross production values 20% higher than ^{14}C results corrected for filtration error.

$\frac{I_{od}}{I_{oe}}$ as a multiplication factor, where I_{od} is the full-day incident solar radiation and I_{oe} is the incident radiation during exposure of the production bottles. Total CO_2 values were calculated as described in the chemical section of this paper. A conversion factor of 1 g C = 11.25 kcal was used to convert carbon uptake to units of energy for calculating production efficiency, assuming a PQ (photosynthetic quotient) of 1 and a calorific value of 4.5 kcal/g for carbohydrate.

On each date phytoplankton samples of 0.8 liters were taken at 1-m and at 10-m depths, as well as with a 10-m length of plastic tubing lowered vertically into the lake (Lund and Talling, 1957). Samples were preserved in acid Lugol's solution, and concentrated by settling in long tubes into 10-ml counting cells. Counts were made with a 20× phase-contrast objective and a calibrated 10× eyepiece on an inverted microscope. For identification purposes 20-m vertical hauls were taken with a 10-μ nannonet and examined fresh in a nannochamber on the inverted microscope. All cell counts were converted to cell volumes, using conversion factors from Nauwerck (1963) or from the data of Dr S. Holmgren of the Fisheries Research Board of Canada Freshwater Institute, Winnipeg (manuscript in preparation).[5] The taxonomic works of Prescott (1961), Bourrelly (1966), Huber-Pestalozzi (1938, 1941, 1942, 1950), and Skuja (1948, 1956, 1964) were used for identification of the phytoplankton. The classification system of Huber-Pestalozzi was followed.

On each date 500–1000 ml of water from just below the surface were filtered onto a millipore HA membrane for chlorophyll analyses. The procedure of Strickland and Parsons (1965) was followed. Readings were made in 10-cm small-volume spectrophotometer cells.

Chemical analyses — Summer oxygen concentrations were measured with a Sargent "Precision" galvanic-cell oxygen electrode, calibrated by the Winkler method. The standard Winkler technique was used in winter. Temperature was measured with the thermistor contained in the oxygen measuring unit, or with a thermistor manufactured by Applied Research Associates of Texas. Accuracy was about ±0.5 C.

Transparency readings were taken with a 35-cm white Secchi disc. Submarine light measurements were made with a Gemware submersible light meter (barrier-layer photocell) on several occasions. A Belfort model 5-3850 pyrheliograph was used to measure incident solar radiation.

The *p*H was measured from water collected in standard Biological Oxygen Demand bottles. A 100-ml aliquot from the same bottle was titrated with 0.005 N HCl to estimate total CO_2, with a *p*H meter to determine the endpoint.[6] The dissociation constants given by Garrels and Christ (1965) were used, and the activity coefficients for H_2CO_3, HCO_3^-, and $CO_3^=$ were assumed to equal unity in these dilute waters. Because of the low CO_2 concentrations and the high proportion of free CO_2, midsummer measurements of total CO_2 may be as much as 10–15% in error, as determined by replicate titrations, representing the greatest single source of error in applying the ^{14}C method to Shield waters (Schindler and Holmgren, 1971). Major cations were measured on two dates by atomic absorption or flame photometry; the ion-exchange methods of Mackereth (1963) were used for sulfate and chloride measurements.

The methods outlined by Strickland and Parsons (1965) were used for all analyses for nitrogen, phosphorus, and silicate, except that ammonia was measured as described by Strickland and Parsons (1960). All nutrient samples were collected at 5-m vertical intervals with a van Dorn bottle, stored in polyethylene bottles, and frozen unfiltered. Occasional runoff, outflow, and precipitation samples were treated in the same manner. Noncorrosive chemicals used in nutrient analyses were conveniently dispensed from Cornwall automatic pipettes connected to 1-liter aspirator bottles with tygon tubing. A Hitachi-Perkin Elmer EPS-3T spectrophotometer with 1-, 5-, or 10-cm cells was used for all absorptiometric work.

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[5]Cell volumes for species in Clear Lake were very similar to those found by the above authors (S. Holmgren, personal communication).

[6]The inflection point in a plot of ml titrant vs. Δ *p*H was used as an endpoint. The bicarbonate endpoint in Shield waters is variable (*p*H 4.5–5.2) and titration to a set *p*H should be avoided. Schindler and Holmgren (1971) give other objections to the titration procedure in dilute waters.

Hydrological budget, chemical input, and weathering by solution — Since precipitation recorded at three Canada Department of Transport meteorological stations within 20–25 miles of Clear Lake differed by only a few percent, data from the nearest station, Haliburton, were used here. Average lake evaporation was estimated from the map of Bruce and Weisman (1967), and data from Ottawa, the nearest Canada Department of Transport station making such measurements, were used for calculations pertaining to the period of observation. Average evapotranspiration was estimated by the Canada Land Inventory (1966) using the method of Thornthwaite (1948). For the period of study, evapotranspiration was estimated to be 0.9 times lake evaporation (Department of Transport, personal communication). These data are as follows (in meters):

	Rain (P_R)	Snow (P_S)	Total precipitation (P_{R+S})	Lake evaporation (E)	Evapotranspiration (E_t)
Annual avg	0.700	0.204	0.904	0.635	0.530
Apr. 1967– Mar. 1968	1.034	0.229	1.263	0.646	0.581

The water income (I) to the lake was assumed to be

$$I = A_o(P_{R+S}) + A_d(P_{R+S} - E_t) \quad (1)$$

and outflow (L) was assumed to be

$$L = I - A_oE \quad (2)$$

so that the annual water renewal rate (τ) equalled

$$\tau = V/L. \quad (3)$$

These calculations are as follows (m^3/year):

	Precipitation ($A_o(P_{R+S})$)	Runoff ($A_d(P_{R+S}-E_t)$)	Total water income (I)	Evaporation (A_o)(E)	Outflow (L)	Annual water renewal rate (τ)
Long-term annual avg	799,000	468,000	1,267,000	561,000	706,000	0.06
Apr. 1967–Mar. 1968	1,116,000	852,000	1,968,000	571,000	1,398,000	0.13

The following equations were used to calculate the mass of any ion in income or outflow water:

$$M_R = A_o\, C_R\, P_R \quad (4)$$
$$M_S = A_o\, C_S\, P_S \quad (5)$$
$$M_T = A_d(P_{R+S} - E_t)\ (C_T) \quad (6)$$
$$M_I = M_P + M_S + M_T \quad (7)$$
$$M_L = C_L\,(A_o(P_{R+S} - E) + A_d(P_{R+S} - E_t)) \quad (8)$$

where M_R, M_S, M_T, and M_L represent the total mass of ion entering or leaving the lake as rain, snow, runoff, and outflow, and C_R, C_S, C_T, and C_L represent the concentrations of ions in rain, snow, runoff, and outflow. Groundwater was ignored in this estimate, since soils and tills are thin or absent and the excess of precipitation over evapotranspiration is not high. There are no obvious places (e.g., faults in the bedrock) where seepage to other lakes could occur.

A high proportion of the runoff from the terrestrial part of the watershed occurs during or immediately after snowmelt.

RESULTS

Primary production and phytoplankton — Carbon uptake was usually maximum at a depth of 2–3 m (Fig. 3). Photoinhibition was common at the

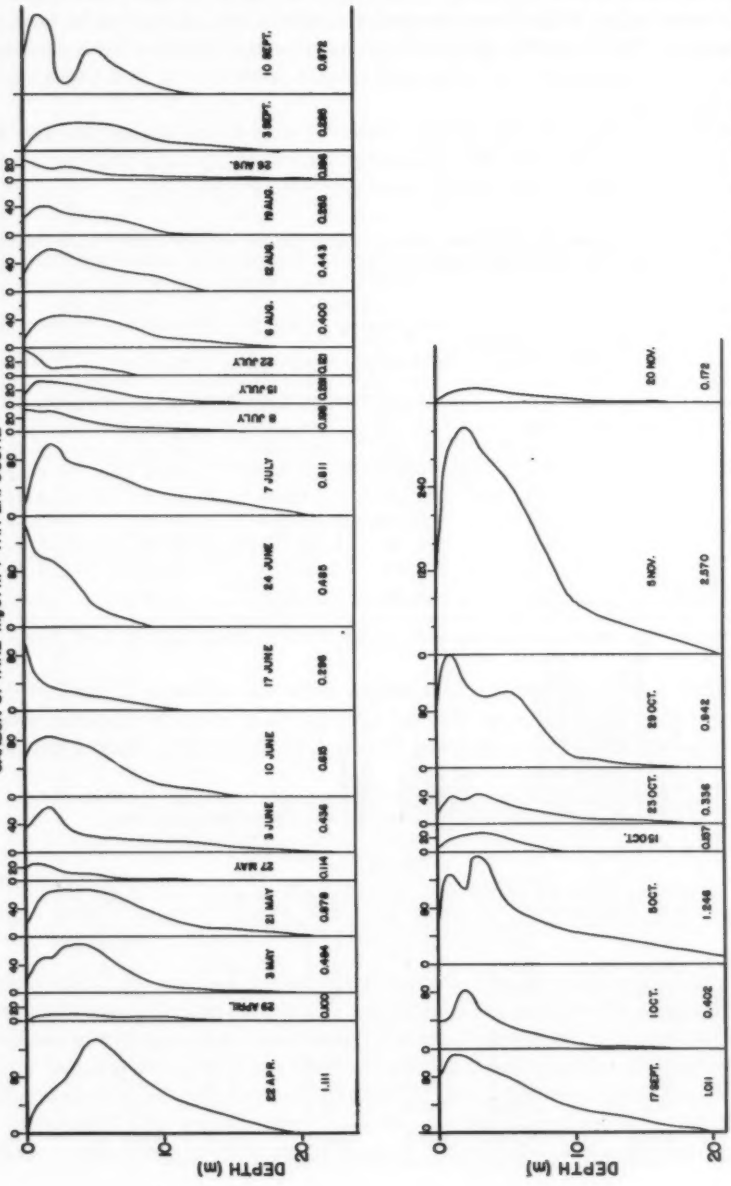


FIG. 3. Primary production profiles for Clear Lake, 1967. Values shown are corrected for filtration error as described by Arthur and Rigler (1967). Numbers at bottoms of panels are g C/m³ per 4-hr exposure (10:00 AM–2:00 PM).

surface on almost all dates, and daylight compensation depths were usually near 20 m, where light intensity was approximately 1% (Fig. 10 and the section on chemical and physical observations). The highest photosynthetic rates were measured just after ice left the lake and again in late fall. Production values were higher than those previously reported for other lakes in the Canadian Shield. This is partly due to the correction for filtration error mentioned above, which averaged 3.2 times and ranged from 2.7 to 3.6 times the uncorrected values.

Highest production efficiencies (Table 1) were found during the fall overturn, when NO_3, CO_2, and PO_4 concentrations were all high. High production also occurred during the spring overturn period (Fig. 3).

TABLE 1. Monthly production efficiencies for a water column in Clear Lake during the ice-free season, 1967. Corrected values include the correction for filtration error (Arthur and Rigler, 1967).

	I_o during exposure (10^4 gcal/m^2)	cal/m^2 fixed during exposure		% efficiency (=production/incident light)	
		Uncorrected	Corrected	Uncorrected	Corrected
Apr.	404	3,940	12,610[a]	0.10	0.31
May	446	5,188	16,600[a]	0.12	0.37
June	620	6,394	20,460[a]	0.10	0.33
July	721	5,488	17,560[a]	0.08	0.24
Aug.	338	5,189	14,010	0.15	0.42
Sept.	354	8,100	27,540	0.23	0.78
Oct.	526	15,488	52,660	0.29	1.00
Nov.	317	11,003	39,610	0.35	1.25
Total or mean	3,726	60,790	201,050	0.18	0.59

[a]The average filtration correction from August to November is used for April–July results.

Table 2 combines production values with the volumes of various depth profiles within Clear Lake, so that an average value of phytoplankton production per unit area may be obtained for the lake as a whole. Such a procedure

TABLE 2. Total phytoplankton production (10^6 kcal) in Clear Lake, 1967. Data are weighted for volumes of different strata.

	Depth (m)				
	0–5	5–10	10–15	15–20	Total
Apr.	96.4	76.9	18.3	1.8	193.4
May	238.4	80.8	21.5	2.9	343.6
June	221.0	52.7	14.3	2.4	290.4
July	163.1	50.7	12.6	1.4	227.8
Aug.	220.2	75.9	24.3	2.1	322.5
Sept.	340.7	103.0	33.8	8.3	485.8
Oct.	243.4	79.8	17.0	7.2	347.4
Nov.	177.6	66.8	22.3	3.5	270.2
Total	1700.8	586.6	164.1	29.6	2481.1

seems justified in this lake, where littoral macrophytes are almost completely absent, and is necessary when efficiency of production by zooplankton is considered (Schindler, 1971, and in preparation).

Figures 4–6 show seasonal changes in chlorophyll and common phytoplankton. *Peridinium* spp. (chiefly *inconspicuum*, *willei*, and *wisconsiense*) were common in winter and early spring. Once ice left the lake, *Synedra* spp.,

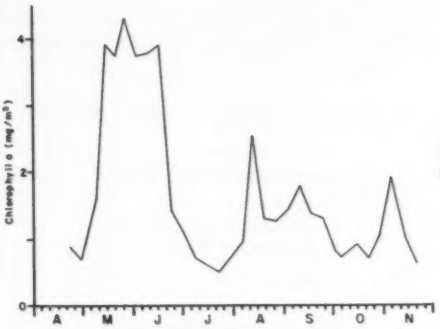
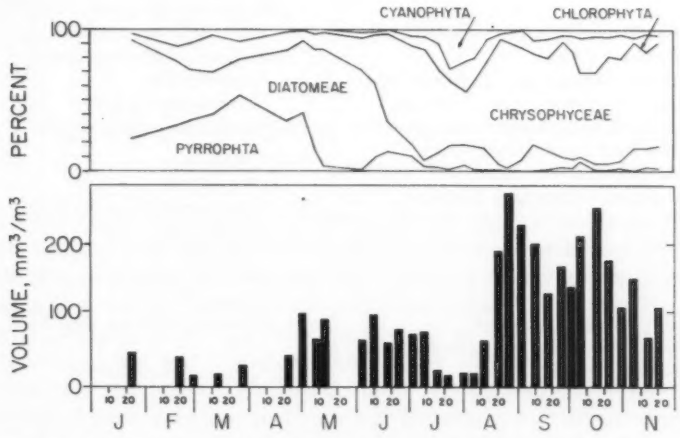


FIG. 4. Seasonal changes in chlorophyll *a* concentrations in the euphotic zone, 1967.



FIGURES 5 AND 6. Fig. 5 (*above*): Percent compositions (by volume) of phytoplankton by major groups, 1967. Fig. 6 (*below*): average phytoplankton volumes in the euphotic zone, 1967.

Asterionella formosa, and *Tabellaria fenestrata* became common. The colonial Chrysophyceae *Synura uvella*, *S. adamsii*, *Chrysosphaerella longispina*, and *Uroglena americana* were common in midsummer, making up a high proportion of the biomass because of the large volume of their colonies. A pulse of *Volvox tertius* occurred in late July. In late summer, *Dinobryon* spp. (*bavaricum*, *sertularia*, *divergens*, and *cylindricum*) joined the above-mentioned colonials. The species composition is typical for a small Shield lake, although smaller forms

were probably missed because of the low counting magnification. Data for 1968 showed similar species composition and succession although the times of occurrence of blooms were slightly later. Both cell volume and chlorophyll concentrations were extremely low, but within the range expected for an oligotrophic lake. Of particular interest is the presence of small numbers of *Fragilaria crotonensis*, which Stockner and Benson (1967) consider to be an indicator of incipient eutrophication. Although this fact is difficult to reconcile with optical and chemical properties of the lake, it may be connected with the remarkably high production observed. A list of phytoplankton species for one midsummer date is given in Table 3.

TABLE 3. Clear Lake, common phytoplankton, July 2, 1968.

1. Cyanophyta
   - *Aphanothece clathrata* W. & G. S. West
   - *Chroococcus limneticus* Lemm.
   - *Gloeothece linearis* Naegeli
   - *Merismopedia tenuissima* Lemm.
   - *Rhabdoderma lineare* Schmidle & Lauterborn

2. Chlorophyta
   - *Ankistrodesmus falcatus* var. *acicularis* (A. Braun) West
   - *Crucigenia rectangularis* (A. Braun) Gay
   - *Gloeococcus schroeteri* (Chod.) Lemm.
   - *Oocystis lacustris* Chodat (atypical)
   - *O. submarina* var. *variabilis* Skuja
   - *Pleodorina californica* Shaw
   - *Staurodesmus triangularis* (Lagerh.) Teiling
   - *Volvox tertius* Meyen

3. Chrysophyta
   - Chrysophyceae
     - *Dinobryon bavaricum* Imhof.
     - *D. cylindricum* Imhof. var. *palustre* Lemm.
     - *D. divergens* Imhof.
     - *Mallomonas caudata* Iwanoff.
     - *Synura uvella* Ehr.
   - Chysophyta (*Cont.*)
     - Heterokontae
       - *Botryococcus braunii* Kütz.
       - *B. protuberans* W. & G. S. West

4. Diatomeae
   - *Cyclotella comta* (Ehr.) Kütz.
   - *Melosira italica* (Ehr.) Kütz.
   - *Stephanodiscus astraea* (Ehr.) Grun.
   - *Asterionella formosa* Hass.
   - *Fragilaria crotonensis* Kitton
   - *F. pinnata* Ehr.
   - *Navicula pupula* Kütz.
   - *Synedra ulna* (Nitsch.) Ehr.

5. Pyrrophyta
   - Cryptophyceae
     - *Cryptomonas marssonii* Skuja
     - *C. ovata* Ehr.
   - Dinophyceae
     - *Ceratium hirundinella* (O. F. Muell.) Dujardin
     - *Peridinium cinctum* (Muell.) Ehr.
     - *P. inconspicuum* Lemm.
     - *P. willei* Huitf-Kass
     - *P. wisconsiense* Eddy

Chemical, biological, and physical observations — Oxygen in the lower hypolimnion was reduced to about 20% saturation by midsummer (Fig. 7), although temperatures in deep water did not rise above 5 C (Fig. 8). The midsummer supersaturation with oxygen in the upper hypolimnion was due to the shallow thermocline, high transparency, and relatively high primary production. Circulation was complete in both spring and fall of both years observed. Because of heavy snow cover, ice thickness was seldom greater than 0.5 m (3 years' observations), although a slow depletion of oxygen beneath the ice confirmed ^{14}C measurements in indicating that winter photosynthesis

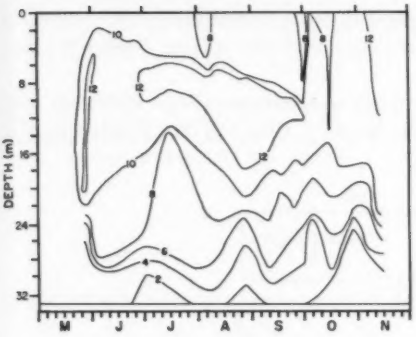


FIG. 7. Seasonal oxygen isopleths, ppm, 1967.

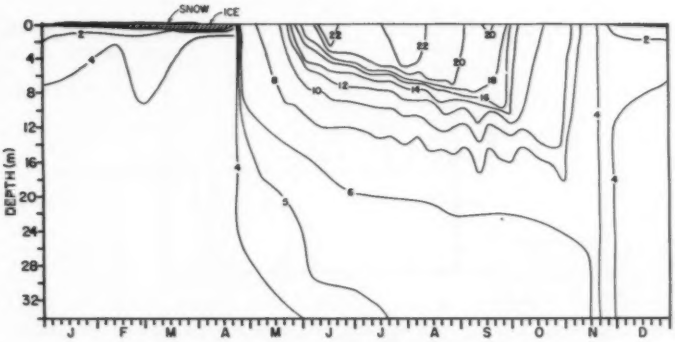


FIG. 8. Seasonal isotherms, C, 1967.

was negligible under snow cover, despite relatively high populations of apparently healthy algae. These algae were usually in the coldest water directly under the ice, and probably remained in a state of near dormancy, possibly maintaining population levels by dark fixation even though they possessed chlorophyll (Wright, 1964). Dr S. K. Holmgren of the Freshwater Institute (personal communication) found similar healthy phytoplankton populations under ice and snow in Swedish lakes and in the Fisheries Research Board Experimental Lakes Area in northwestern Ontario.

The lake is very clear, with Secchi disc visibility usually greater than 10 m (Fig. 9). Because of the low total CO_2, high photosynthesis, and low

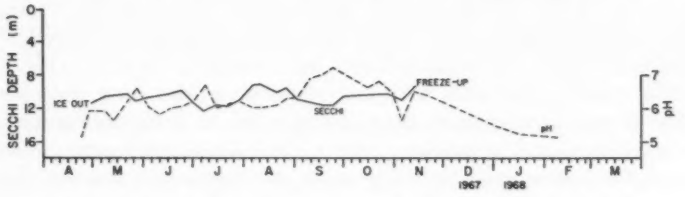


FIG. 9. Seasonal changes in *p*H and Secchi disc visibility, 1967.

buffering capacity of the water, a marked diurnal variation in *p*H, up to a full *p*H unit, was observed on some occasions in early spring. Figure 9 gives seasonal *p*H values.

Light penetration was extremely high in summer (Fig. 10) with 1% of surface intensity occurring at depths greater than 20 m. Winter values

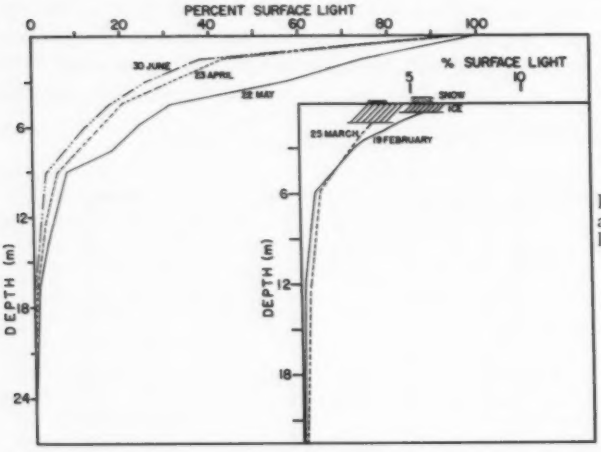


FIG. 10. Light penetration as percent of surface illumination on representative dates.

shown were obtained late in the season. In midwinter, when 20–50 cm of wet snow covered the lake, well under 1% of incident light reached the lower surface of the ice.

Concentrations of major ions in surface waters of the lake (meq/liter, May 1968) were:

Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Total	HCO_3^-	$SO_4^=$	Cl^-	Total
0.125	0.057	0.032	0.012	0.226	0.033	0.168	0.028	0.229

The high sulfate, calcium, and magnesium with respect to sodium, potassium, bicarbonate, and chloride appear to typify inland Shield waters in well-scoured bedrock areas, although the sulfate value is the highest we have encountered in the area. Clear Lake's conductivity (30 μmhos/cm, 25 C) is about average for small lakes in the area (range for eight lakes = 20–55 μmhos/cm at 25 C). The difference between the curves for bicarbonate and total CO_2 values was due to free CO_2 at the low *p*H values encountered (Fig. 11).

Nitrate-nitrogen, orthophosphate, and reactive silicate (Fig. 12–14) had similar seasonal patterns, with low midsummer and early autumn values in surface waters accompanied by some increase in the hypolimnion during summer stratification. The amount present in the hypolimnion was not sufficient to account for losses from the epilimnion, so that sedimentation (probably by organic processes) occurred all summer. Nutrient regeneration from the

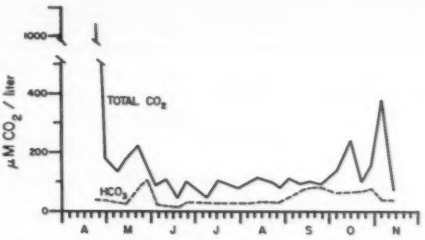
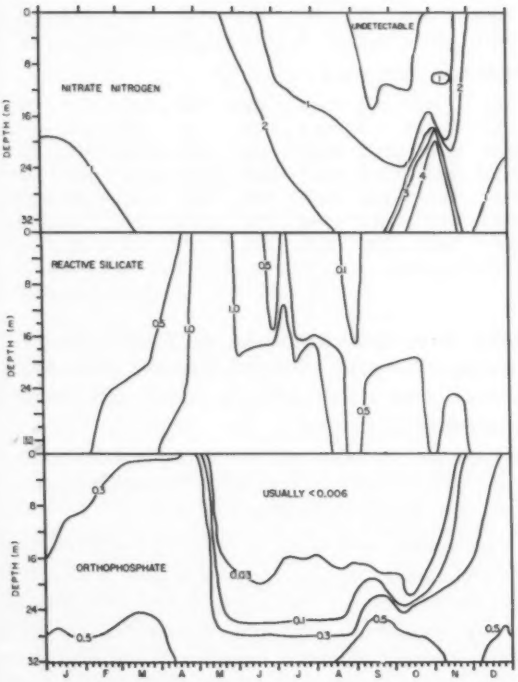


FIG. 11. Seasonal changes in bicarbonate and total CO_2, μmoles/liter, 1967. The difference between the two curves is due almost entirely to free CO_2.



FIGURES 12–14. Seasonal changes, 1967: Fig. 12 (*top*): NO_3-N (μg-at/liter); Fig. 13 (*middle*): reactive Si (mg/liter); Fig. 14 (*bottom*): PO_4-P (μg-at/liter).

sediments did not appear to contribute significantly to the yearly nutrient budget of the lake, contrary to the findings of Hynes and Greib (1970) for southern Ontario lakes. The major rejuvenation of yearly nutrient concentrations in lake water appeared to occur during the period of snowmelt in the spring.

Chemical budgets — Table 4 gives chemical concentrations of terrestrial runoff, rain, snow, and outflow (C_T, C_R, C_S, and C_L). Since chemical concentrations could not be weighted for seasonal differences in inflow and outflow volumes, only the grossest features of the chemical budgets are of interest.

TABLE 4. Representative chemical analyses of inflow, outflow, rain, and snow (μg/liter), Clear Lake. April samples were taken during the period of maximum runoff from snowmelt, June samples during moderate runoff from snowmelt and spring rains, July and August samples during minimum flow.

	Inflow											
	West inflow stream				E side of basin	Outflow			Rain		Snow	
	Apr.	June	July	Aug.	Apr.	Apr.	June	July	June	July	Jan.	Feb.
NH_3-N	345	180	165	–	–	0	0	0	550	560	–	625
NO_3-N	200	44	38	45	215	21	27	10	210	185	430	210
Total P	36	21	17	12	31	15	7	7	19	23	–	60
Total dissolved P	5	6	4	4	4	4	–	4	18	17	9	12
PO_4-P	4	2	2	2	3	9	<1	<1	4	4	6	4
Reactive Si	1975	450	425	440	1850	1050	750	485	252	216	106	115
Ca	1800	1910	1630	1790	1890	1710	1810	1600	1100	1310	690	760
Mg	600	550	640	620	710	700	640	710	450	550	90	110
Na	390	450	470	390	410	670	700	760	100	75	170	150
K	190	200	250	190	260	380	410	410	85	70	80	50

As described earlier, precipitation in the year beginning April 1967 was 40% greater than normal, mainly because of heavy rains in May and June. Since evapotranspiration and evaporation were nearly normal, runoff and outflow were nearly double the average values.

Table 5 gives chemical budgets for the lake and for the terrestrial watershed, based on long-term average hydrological parameters. The same calculations, using data from 1967–68, are shown in Table 6.

Of the major ions, sodium, potassium, and silica were much more concentrated in runoff than in precipitation, indicating that they are largely weathered from the drainage basin. High percentages of the calcium and magnesium in precipitation were retained by terrestrial soils and vegetation, so that the supply to the lake was nearly equally balanced between runoff and precipitation directly on the lake surface. Concentrations of both elements are high compared to values in the Fisheries Research Board Experimental Lakes Area of northwestern Ontario (F. A. J. Armstrong, J. Barica, and D. W. Schindler, unpublished data).

Precipitation supplies most of the nutrients phosphorus and nitrogen to Clear Lake. Both the lake and the terrestrial watershed retained high proportions of the nutrient supplied, presumably because of biotic uptake. Gorham (1958) found that atmospheric precipitation supplied a major part of the inorganic nitrogen received by lakes in the English Lake District. Fisher et al. (1968) calculated that precipitation supplied most of the inorganic nitrogen to Hubbard Brook, New Hampshire.

TABLE 5. Yearly balances (kg) of several ions for Clear Lake and the terrestrial watershed on basis of long-term average hydrological data.

Source[a]	Ca	Mg	Na	K	Total P	Dissolved Si	Dissolved inorganic N
Clear Lake							
M_R	745	309	54	48	13	145	466
M_S	131	18	29	12	11	20	170
M_T	844	292	197	102	11	481	158
M_I	1720	619	280	162	35	646	794
M_L	1205	482	501	282	7	538	13
Net retention	515	137	−221	−120	28	108	781
% of supply retained	30	22	–	–	80	17	98
Terrestrial watershed							
Rain	1054	438	76	68	18	205	658
Snow	181	25	40	16	15	28	236
Total precipitation	1235	463	116	84	33	233	894
M_T	844	292	197	102	11	481	158
Net retention	391	171	–	–	22	–	736
Net loss	–	–	81	18	–	248	–
% of supply retained	32	37	–	–	67	–	82
Weathered (*kg/ha*)	–	–	0.65	0.14	–	1.98	–
Retained (*kg/ha*)	3.12	1.37	–	–	0.18	–	5.89

[a] M_R = direct rain; M_S = direct snow; M_T = terrestrial runoff (total runoff from rain and snow for terrestrial watershed); M_I = total input; M_L = outflow.

GENERAL DISCUSSION

Chemical budgets for terrestrial drainage — The high rates of weathering by solution of sodium, potassium, and silica from the terrestrial watershed in relation to those for calcium and magnesium would be expected if the bedrock in the Clear Lake watershed is of similar composition to that of other areas in Canadian Shield (Brunskill et al., 1971). Nevertheless, it is difficult to explain why runoff of calcium and magnesium from the terrestrial area is less than that supplied by the atmosphere (Table 5, 6).

Phosphorus and nitrogen are even more strongly retained by the terrestrial drainage. If the terrestrial ecosystem is in some sort of dynamic equilibrium with the nutrient supplied by atmosphere, nutrient output from the ecosystem should equal input, i.e., nutrient released by decomposition in vegetation and soils should equal that entering as precipitation.

Several factors may be responsible for the above discrepancies. Matheson (1951) found that 39% of atmospheric nitrogen supplied to a non-Shield portion of Ontario was in the form of dry atmospheric fallout, which was largely ignored in this study.

No analyses of nitrogen in organic form, either dissolved or particulate, were done in this study. Probably, efficient conversion of the inorganic forms

TABLE 6. Yearly balances (kg) of several ions for Clear Lake and the terrestrial watershed on basis of hydrological data for the year beginning April 1967.

Source[a]	Ca	Mg	Na	K	Total P	Dissolved Si	Dissolved inorganic N
			Clear Lake				
M_R	1101	457	80	71	19	214	688
M_S	147	20	32	13	12	22	191
M_T	1537	531	360	186	20	876	288
M_I	2785	1008	472	270	51	1112	1167
M_L	2386	955	993	559	14	1065	27
Net retention	399	53	−521	−289	37	47	1140
% of supply retained	14	5	–	–	73	4	98
			Terrestrial watershed				
Rain	1557	646	114	101	27	302	973
Snow	208	29	46	19	17	31	271
Total precipitation	1765	675	160	120	44	333	1244
M_T	1537	531	360	186	20	876	288
Net retention	288	144	–	–	24	–	956
Net loss	–	–	200	66	–	543	–
% of supply retained	13	21	–	–	55	–	77
Weathered (*kg/ha*)	–	–	1.6	0.5	–	4.3	–
Retained (*kg/ha*)	2.3	1.2	–	–	0.2	–	7.6

[a]For abbreviations see footnote a of Table 5.

of nitrogen that predominate in rain to organic forms by the terrestrial biota caused the element to be underestimated in runoff. The possibility of denitrification cannot be overlooked.

If the concentrations of ions in precipitation and runoff vary little from year to year, an increase of 25% in the annual precipitation could cause a twofold increase in runoff of ions from the basin (cf. Table 5, 6). The possible consequences of increased nutrient loading for the lake are discussed below. More data would be needed for an accurate calculation of the effect of higher than average annual precipitation, since Johnson et al. (1969b) found that sodium and silica concentrations in runoff were diluted during periods of high flow, nitrate concentrations increased in proportion to flow, and concentrations of calcium and potassium remained relatively constant.

Chemical budgets for the entire watershed — The lake appeared to be as effective as or more effective than the terrestrial basin at retaining calcium and magnesium, depending on whether precipitation was average or greater than average (Table 5, 6). Nitrogen and phosphorus were also retained more effectively by the lake. Since the quantities of nitrogen and phosphorus dissolved in the lake were very low (e.g., 2–3 kg of P for the whole lake), a loss by sedimentation is presumed.

Comparison of chemical losses with those in other watersheds — Losses of major ions from the total watershed of Clear Lake are slightly higher than

for lakes in the Fisheries Research Board Experimental Lakes Area in northwestern Ontario, and also in the Canadian Shield (Table 7). Hubbard Brook, New Hampshire, a drainage underlain by marble, has greater losses of calcium, but is otherwise quite similar. Losses of nitrogen from both Hubbard Brook

TABLE 7. Net chemical losses (kg/ha per year) from four watersheds in ancient rock.

Clear Lake				Fisheries Research Board Experimental Lakes Area (including lake)	
Terrestrial watershed	Total watershed (including lake)	Hubbard Brook, N.H. (terrestrial watershed)	Central Sweden River Basin (terrestrial watershed)	Lake 239	Lake 240
Neg.[a]	5.6[b]	10.6	–	1.9[b]	1.6[b]
Neg.	2.3[b]	2.5	–	0.7[b]	0.6[b]
0.65	2.3	6.1	–	1.0[b]	1.1[b]
0.14	1.3	1.5	–	0.5[b]	0.4[b]
2.0	2.5[b]	–	–	1.4[b]	1.8[b]
1.3[b]	0.06[b]	3.3–4.5[c]	0.65–1.75		
0.09[b]	0.03[b]	–	0.03–0.06		
Present study		Borman et al. (1968) Johnson et al. (1968) Likens et al. (1969)	Brink (1964, cited by Vollenweider, 1968a)	Brunskill et al. 1971	

[a]Neg. = negative.
[b]Input to the system exceeded output.
[c]Dissolved inorganic only.

and a river basin in central Sweden are somewhat higher than the total Clear Lake watershed, but are similar to those for the terrestrial watershed alone, indicating effective nutrient retention by the lake.

Relation of phytoplankton production to chemical regimes and other environmental factors — The oxygen regime (Fig. 7) for Clear Lake is not one to be expected from a highly productive lake of such morphometry. The absence of a large summer oxygen deficit may, however, be because highest production occurs during spring and fall turnover (Fig. 3, 8), when any decomposition would not be expected to contribute to the hypolimnetic oxygen deficit.

Highest production occurred in spring and late fall, when nutrients were highest, rather than in late June, when light was optimal, implying that production in Clear Lake is nutrient limited. The maximum concentrations of the nutrients NO_3-N and PO_4-P corresponded with those of CO_2 (Fig. 12, 14). Johnson et al. (1969b) found that CO_2 limited algal production in the three Shield lakes mentioned above. Sakamoto (1971) found that all three substances may limit production in particular situations in Shield lakes. An attempt to analyze these relations was made by regressing areal phytoplankton production (Pr, in g C/m² per 4-hr exposure) upon concentrations of the nutrients inorganic nitrogen (N, μg/liter) and total CO_2 (CO_2, μmoles/liter), as well as water temperature (T, C), solar radiation (I_o, gcal/m² per 4-hr exposure), and chlorophyll concentration (Chl, μg/liter), using multiple linear

regression analysis (Table 8). Phosphate was not included because of the high frequency of undetectable values, and because its high turnover (Rigler, 1964) makes its concentration meaningless as a measure of availability to phytoplankton.

TABLE 8. Results of the multiple linear regression analysis relating Clear Lake phytoplankton production to environmental factors during the ice-free season.

Pr = 326.04 I_o	+ 180.14 Chl	+ 28.98 Si	− 63.30 N	− 156.89 CO_2	− 0.722 T	− 189
SE of partial regression coefficient						
64.28	46.20	10.91	25.00	125.24	0.385	
t-value of partial regression coefficient						
5.07(HS[a])	3.90(HS)	2.66(HS)	−2.53(HS)	−1.87(NS[a])	−1.26(NS)	
Standard partial regression coefficient						
1.37	0.50	0.48	0.43	0.27	0.40	
Coefficient of Multiple Determination, R^2 = 0.66						
F value = 7.63(HS)						
df = 6 and 24						

[a]HS = highly significant; NS = not significant.

Covariation among nitrate, silicate, and CO_2 appeared to obscure the possible relation between any of the three nutrients and phytoplankton production, and it is impossible to tell which of the three substances might be the primary limiting factor. Solar radiation, chlorophyll, silicate, and nitrate contributed significantly to regression (Table 8), yet when silicate and nitrate were omitted, light and CO_2 explained almost as large a proportion of the variance in production (R^2 = 0.63). All partial regressions were tested for linearity (Ezekial and Fox, 1961), but no significant departures were detected.

Phytoplankton production calculated from the regression equation compared favorably with that actually measured in the lake (Fig. 15). This

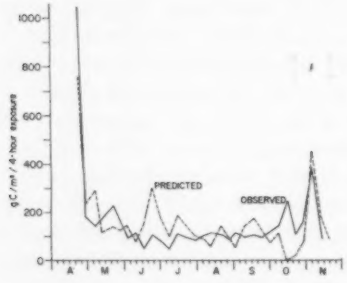


FIG. 15. Phytoplankton production in 1967 as observed and as "predicted" by multiple regression. Exposures from 10:00 AM to 2:00 PM.

technique has been used successfully by several authors (Johnson et al., 1969b; Goldman et al., 1968; Schindler, MS, 1966; Schindler and Comita, 1971; Comita and Wolf, 1967). Although the results given by some of the above

authors do not allow detailed interpretation,[7] a general comparison is possible (Table 9).

The independent variables important as predictors of phytoplankton production were similar in most studies for which data were available (Table 9). Chlorophyll or phytoplankton were important in all five of the studies reviewed, temperature and light in four, dissolved inorganic nitrogen and carbon in three, and silicates in two of five.

Phytoplankton production in Clear and other Shield lakes — Clear Lake is more productive than any other lake reported in the Canadian Shield (Clear Lake average for ice-free season = 1020 mg C/m² per day). Johnson et al. (1969b) reported mean midsummer production rates for Dunlop, Quirke, and Pecors lakes, near Sudbury, Ont., of 126, 71, and 34 mg C/m² per day, respectively. These averages do not include the spring and fall overturn periods, which may be more productive. The above authors found evidence for carbon limitation in the lakes, two of which were polluted with acid uranium-processing wastes. They did not apply the correction for filtration error.

Small lakes (2–1000 ha) in the Fisheries Research Board Experimental Lakes Area near Kenora, Ont., have midsummer production averaging 200–700 mg C/m² per day (Schindler, 1971; Schindler and Holmgren, 1971).

Production in Scandinavian lakes — Rodhe (1958) and Findenegg (1964) gave production values for lakes with temperature regimes, optical properties, and geological substrates similar to Clear Lake. Production values were considerably lower than ours. Again, the fact that data uncorrected for filtration error may be 1.3–5.6 times too low (Schindler, 1971; Schindler and Holmgren, 1971) may be the explanation.

Other highly productive transparent waters — Only Baranov (1959) reports areal production values for transparent lakes (Karelian lakes, northwestern USSR) that are comparable to those for Clear Lake. His measurements were done with the oxygen bottle method. The lakes have similar nutrient properties, although major ion concentrations in the Karelian Lakes are slightly higher (Baranov, 1962). The maximum production reported by Baranov (summarized by Winberg, 1963) was 5.4 g O_2/m² per day (gross production), from Lake Elmozero. Since roughly 18% of this was reported to be respiration, net production was 4.4 g O_2/m² per day, or about 1.7 g C/m² per day, a value not

[7]Some of the above authors mention only variables that contributed significantly to regression in their study, and it is not clear whether other variables were not tried, or were rejected because of their poor predictability. Other workers used logarithmic transformations of all variables before analysis, without any statement about why the transformation was necessary. Another practice in multivariate analyses of production work has been to include a large number of standard limnological measurements as independent variables. Because the direct relation of some of these variables to phytoplankton production is obscure and the probability of a "nonsense" variable obscuring a true causal relation by "masking" an important variable with which it co-varies (the sort of effect mentioned above for nitrate, silicate, and total CO_2), we limit our treatment to variables of known importance to phytoplankton production.

TABLE 9. A comparison of multiple regression analyses relating phytoplankton production to causal factors in several lakes.

Lake	Reference	Order of importance of independent variables						Remarks
		1	2	3	4	5	6	
Severson, 1955	Schindler and Comita (1971)	Temp	Light	Chl	–	–	–	
Severson, 1964	"	Chl	Temp	Dissolved inorganic N	Light	–	–	
Dunlop, Quirke, and Pecors	Johnson et al. (1969)	Inorganic C	Phytoplankton	–	–	–	–	Only two variables mentioned
L. Maggiore	Goldman et al. (1968)[a]	Light	Temp	Silicate	Phytoplankton	Dissolved inorganic N	Inorganic C	No Chl data used
Clear L.	Present study	Light	Chl	Silicate	Dissolved inorganic N	Inorganic C	Temp	

[a]Oxygen, diversity, conductivity, and bacteria are ignored for this comparison.

unlike that of Clear Lake. Average gross production for six large, transparent Karelian lakes was 2.8 g O_2/m^2 per day. Daily respiration on the same lakes averaged 1.15 g O_2/m^2 per day, so average net production was 1.65 g O_2/m^2 per day (approximately 0.62 g C/m^2 per day). In the absence of better data, it is impossible to conclude that Clear Lake is more or less productive than the Karelian lakes.

Winberg (1963) describes a category to include the more transparent Karelian lakes as lakes of secondary (morphometric) oligotrophy or mesotrophy, exhibiting a wide range of production rates (1–7.5 g O_2/m^2 per day and 300–2500 kcal/m^2 per year). These wide limits imply that the category is either a broad one, including lakes of great variety, or an unstable one, with production values varying greatly from year to year. Winberg predicts: "It can be anticipated that in the future the greatest (areal) production of the plankton will be found in lakes belonging to this group due to the most favorable combination of rate of photosynthesis, transparency and depth."

A broader, though less direct comparison of productivity with other waters may be made from a consideration of chlorophyll concentrations and activity coefficients (production/unit chlorophyll), since the activity of phytoplankton pigments must be very high to account for the high production in such transparent waters. The maximum amount of chlorophyll in the euphotic zone of Clear Lake did not exceed 80 mg/m^2, which is well below the theoretical maximum limit calculated by Steemann-Nielsen (1962). Larger quantities have been found in the euphotic zones of both more transparent and more turbid waters. Sakamoto (1966) found a maximum of 106 mg/m^2 in the shallow euphotic zone of eutrophic Lake Suwa. Ichimura (1958) found amounts of up to 200 mg/m^2 in Japanese lakes. Steemann-Nielsen (1962) calculated 129 mg/m^2 in the euphotic zone of Klostersee, studied by Gessner (1943). Winberg (1963) considers that values may be as high as several tenths of a gram per m^2. A comparison of the relative photosynthetic activity of the chlorophyll from these lakes and others is therefore of some interest (Table 10). Spring and autumn Clear Lake activity coefficients are among the highest ever reported, matching those from transparent coastal marine areas. The average value, 5.4 mg C/mg Chl per hr (1.7 if uncorrected for filtration error) is also unusually high if one considers that it represents the entire euphotic zone. Sakamoto (1966) is the only author who has reported average euphotic zone values for several lakes. He reports values ranging from 0.3 to 1.5 mg C/mg Chl per hr. Unfortunately, Table 10 does not include values for the transparent, highly productive Karelian lakes mentioned earlier as we have been unable to locate such information in the literature.

Since areal production values are high, the transformation of solar energy by Clear Lake phytoplankton must be very efficient. The production efficiency for ice-free months averages 0.265% if corrected for filtration error and lake morphometry. Patten (1959) reported a range of values for U.S. lakes of 0.043–0.38%. Hillbricht-Ilkowska and Spodniewska (1969) reported a range of 0.24–0.48% (gross production) for Polish lakes. Their highest efficiencies

TABLE 10. A comparison of hourly production per unit chlorophyll in various types of lakes and other ecosystems.

Lake or area	Author	mg C/mg chl *a* per hr[a]	Method of measurement	Remarks
Several — Japan	Ichimura, 1958	0.64–3.08	O_2 — gross production	Calculated from full-day values
"	Ichimura and Aruga, 1958	0.56–3.41	^{14}C	In laboratory
"	Ichimura and Aruga, 1964	0.1–6	O_2 & ^{14}C	In laboratory at light saturation
Several–Byelorussia	Zakherenkov, 1959	1.2–5.9[b]	O_2	–
Several — Bavaria	Gessner, 1943	0.71–4.37	O_2 — gross production	In laboratory, 10,000 lux
Several — Wisconsin	Manning and Juday, 1941	(2.62)	O_2 — gross production	Optimum light
Several — Japan	Sakamoto, 1966	0.3–1.5[b]	^{14}C	Taken from Fig. 4 Euphotic zone averages
Lake Tahoe, Nevada	Goldman and Carter, 1965	0.3–1.0	^{14}C	Taken from Fig. 3 Euphotic zone averages
Byelorussian ponds	Winberg, 1963	<40[b]	O_2 — gross production	–
Soil vegetation	Rabinowitch, 1951	6–14	–	–
Sea near Madagascar	Sournia, 1968	3–13	^{14}C	–
Sea — Oregon coast	Curl and Small, 1965	6–21	^{14}C	Light saturation
Clear L.	Present study	0.33–21.9 (5.42)[c]	^{14}C	Euphotic zone

[a]Ranges. Values in parentheses are means.
[b]Daily rate ÷ 10.
[c]Mean = 1.69 if data are uncorrected for filtration error.

were obtained in May and October, as in our study. Assuming that net production represents 50–80% of gross, the Clear Lake efficiencies are not exceptionally high.

Interaction of nutrient supply and morphometry — Vollenweider (1969) reports an annual phosphorus supply to Lake Tahoe, Nevada, of 42 mg P/m² per year with an outflow of 3 mg P/m² per year, figures that compare favorably with those of Clear Lake (supply and outflow = 40 and 8 mg P/m² per year, respectively). All other data in the literature are for lakes affected by cultural activities, with much higher nutrient budgets. A comparison of limnological characteristics of Clear Lake and Lake Tahoe (Table 11) is therefore of some interest, even though the lakes are very different. Lake Tahoe is far less productive and more transparent.

TABLE 11. A comparison of limnological features of Clear Lake and Lake Tahoe, Nevada.

	Clear Lake	Lake Tahoe	Unit
Phosphorus supply	40	42	mg/m² per year
Phosphorus outflow	8	3	mg/m² per year
Phosphorus retention	32	39	mg/m² per year
Phosphorus concentration (midsummer avg)	<0.2	7[a]	mg/m³
Secchi disc visibility	8–12	30–40	m
Max midsummer surface temp	22	17	C
Mean depth	12.5	350	m
Depth of euphotic zone	20	60	m
Phytoplankton production	0.10–2.57[b]	0.025–0.24[c]	g C/m² per day
Chl	0.5–4.2	0.06–0.33[c]	mg/m³
Activity index	0.3–21.9	0.3–1.0[c]	mg C/mg chl per hr

[a]The high PO_4-P may be because phytoplankton are limited by nitrogen rather than phosphorus (Goldman and Armstrong, 1969).
[b]Mean = 0.318 g C/m² per day if uncorrected for filtration error, 1.020 if corrected.
[c]Approximate values taken from Fig. 3 of Goldman and Carter (1965).

The difference in the effect of similar nutrient supplies is striking, but not surprising, since the volumes of the lakes are so different. For example, on a volume basis, Clear Lake receives 3.3 mg P/m³ per year; Lake Tahoe receives only 0.12 mg P/m³ per year. The prediction of Vollenweider (1968a) that a given load will have greater effect on a lake with lesser mean depth is supported, though surface area and water renewal time may also be important considerations. Vollenweider's (1968a) calculations would mean that natural sources supply one-fourth of the maximum acceptable phosphorus supply to Clear Lake. Lake Tahoe, with a mean depth 30 times greater, would require a much greater supply of nutrient to reach the same trophic stage (Fig. 16).

Implications for lake management — If Vollenweider's (1968a, b) predictions relating nutrient supply and eutrophication are correct, such parameters

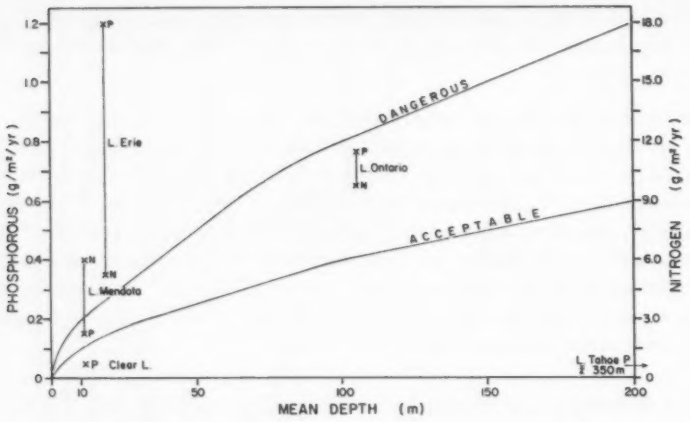


FIG. 16. Phosphorus input (loading) to Clea. Lake and to several well-known North American lakes. Curves represent Vollenweider's (1968a) estimates for permissible and dangerous loading ($\bar{z}$ = mean).

should receive major consideration in planning development within the watershed of any lake. For example, the phosphorus produced as sewage by a human population averages approximately 0.5 kg P/capita-year (Vollenweider, 1968a). Judging by the population and phosphorus-loading figures to the lower St. Lawrence and Great Lakes (International Joint Commission on the Pollution of Lake Erie, Lake Ontario, and the international section of the St. Lawrence River, 1969), the total figure is somewhere between 2.0 and 2.6 kg P/capita-year. From 20 to 80 permanent residents living at the lake's edge could therefore equal the natural nutrient load to Clear Lake. Yet modern lake management, practiced by realtors or regulatory agencies, often encourages the complete colonization of lakeshore areas, and places few restrictions upon disturbance of vegetation and soils on watershed or shoreline. Modern management practice must proceed rapidly to obtain quantitative estimates of nutrient loading and its effect. The desired trophic state of a given lake could then be maintained by regulating the number of dwellings or inhabitants and their locatiohs within a watershed, as well as waste disposal and clearing of natural vegetation.

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Reproduction and Associated Behaviour in the Shorthorn Sculpin, *Myoxocephalus scorpius* in Newfoundland Waters[1]

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In Newfoundland waters, maturing ovaries of the shorthorn sculpin contain three generations of ova. Only the largest or ripe ova of the first generation are deposited at spawning time, which starts in late November or early December and lasts for about 1 month. It appears that only one male and one female participate in the spawning act and the eggs are laid in V-shaped crevices on rocky bottom at depths between 20 and 35 ft (6 and 11 m). The eggs adhere firmly to each other and the egg mass so formed adheres firmly to the bottom. After spawning, the spent females move from the spawning area to deeper water, but the males remain guarding the eggs until they hatch. At temperatures near 0 C embryonic development takes over 3 months to complete. Hatching starts around mid-March and lasts for 3–4 weeks. Newly hatched larvae occupy all layers of the water column over the spawning grounds but tend to concentrate near the bottom. Fecundity of mature females ranging in length from 20.1 to 50.5 cm ranges from 4205 to 60,976 eggs.

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INTRODUCTION

ALTHOUGH the shorthorn sculpin, *Myoxocephalus scorpius* (L.), is common and occurs widely in coastal areas of northern seas (Leim and Scott, 1966), its biology has been little studied. Some work has been published on its biology in the eastern North Atlantic but apart from some casual observations (Bigelow and Schroeder, 1953; Leim and Scott, 1966) nothing has been published on its biology in the western North Atlantic. This paper presents some observations on the reproductive biology and behaviour associated with reproduction of the species in the Newfoundland area of the western North Atlantic.

MATERIALS AND METHODS

Monthly samples of about 20–30 fish were obtained on scuba dives at Bay Bulls (about 20 miles south of St. John's, Nfld.) from October 1965 to April 1966 and from September 1966 to August 1967. They were taken with a hand spear and only a few very small juvenile specimens ever escaped. Ovaries were examined while still fresh under a dissecting microscope, and ova diameters were measured on a 1-mm Turtox grid. Ovaries used for histological sectioning were fixed in Bouin's solution for 1–2 days and kept in about 80% alcohol. Fecundity estimates were

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made for 36 ripe ovaries collected in Freshwater Bay (about 1 mile south of entrance to St. John's harbour) during November 1966 and 1967. To check embryonic development 12 samples of about 200–300 eggs each were taken at 1- to 2-week intervals throughout the winter and early spring from an egg mass that was marked about 2–3 weeks after it was laid. The egg samples were examined after preservation in 10% formalin. Observations were made on the behaviour and activity of adult and newly hatched larval sculpins in their natural environment.

RESULTS

Oogenesis

In a ripening or ripe ovary there are three distinct sizes of ova (Fig. 1A). The largest ova range in size from 0.5 to 0.6 mm in diameter in early summer (June), just before gonad development starts, to about 2 mm in diameter just before spawning in December (Fig. 3). The range in size of these largest, maturing ova is greatest shortly after maturation starts, but near the spawning period the ova are almost uniform in size. These ova are yolk-laden and opaque, and range in colour from pale pink to bright reddish-pink and purplish-pink. As the spawning period approaches, the colour usually becomes more intense, although even some ripe gonads are pale pink. The diameters of medium-size ova range from about 0.3 mm in early summer to about 0.5 mm just before the spawning period. They are usually translucent to opaque in the centre, and around this dark central area is a transparent ring (Fig. 1B). The smallest ova appear during the summer shortly after gonad development starts. They are less than 0.1 mm in diameter during the summer but are about 0.2–0.3 mm in diameter just before the spawning period (Fig. 1C). These ova are completely transparent and will not be mature for another 2 years, so that oogenesis takes about $2\frac{1}{2}$ years. The three sizes of ova are more or less evenly distributed throughout the ovary but only the ripe or largest ova are deposited at spawning time; the others remain intact in the ovary until subsequent spawnings.

Spawning Period

The spawning period of the shorthorn sculpin in this area starts in late November or early December and lasts for about 1 month. On November 30, 1965, one spent female was taken in Bay Bulls when the water temperature was 3.2 C — this is the earliest a spent female was taken. On December 12, 1965, five ripe mature females were taken at Bay Bulls when the water temperature was 4.9 C, but one newly laid egg mass was seen on the same day. On December 21, 1966, two mature females — one ripe and the other spent — were taken at Bay Bulls when the water temperature was 2.7 C — this is the latest a ripe female was taken. On December 28, 1966, three spent mature females were taken at Bay Bulls and about 20 newly laid egg masses were seen the same day.

Spawning

Observations of actual spawning were not made and it is not known if it occurs during daylight or darkness. Probably only one male and one female

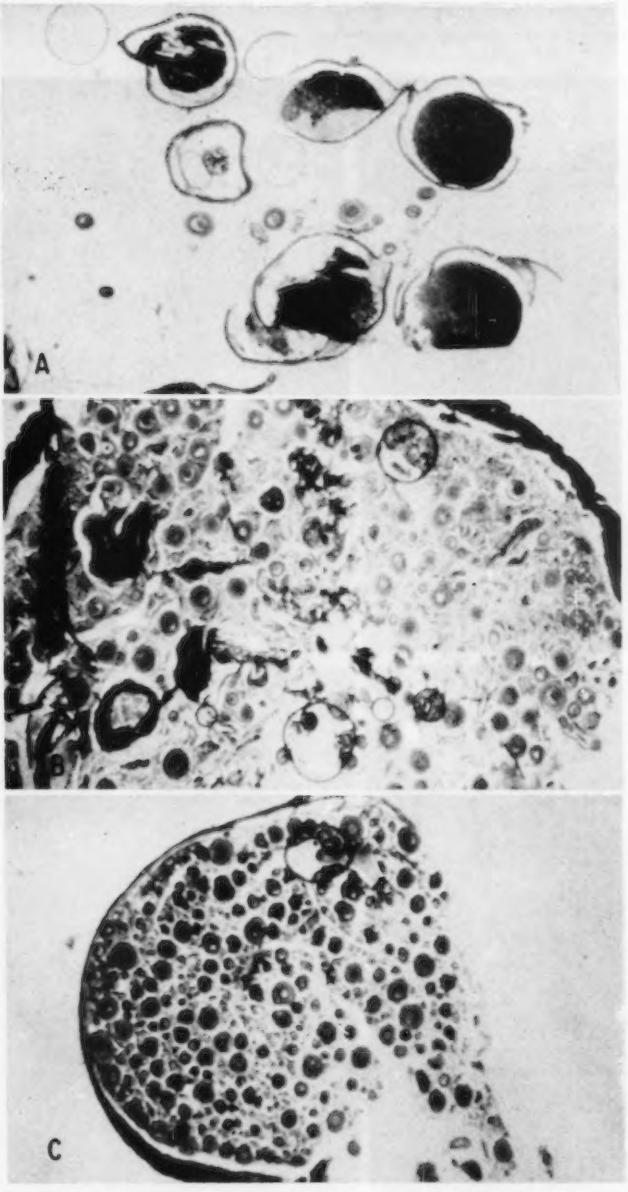

FIG. 1. Sections of ovaries: (A) ripe ovary taken December 21, 1966, at Bay Bulls (note three different sizes of ova); (B) spent ovary taken January 21, 1966, at Bay Bulls (note few residual ova and two other sizes); (C) immature ovary taken November 19, 1966, at Bay Bulls (note two sizes of ova about same as in B. This specimen taken 1 year before it would have matured for first time). All approximately ×10.

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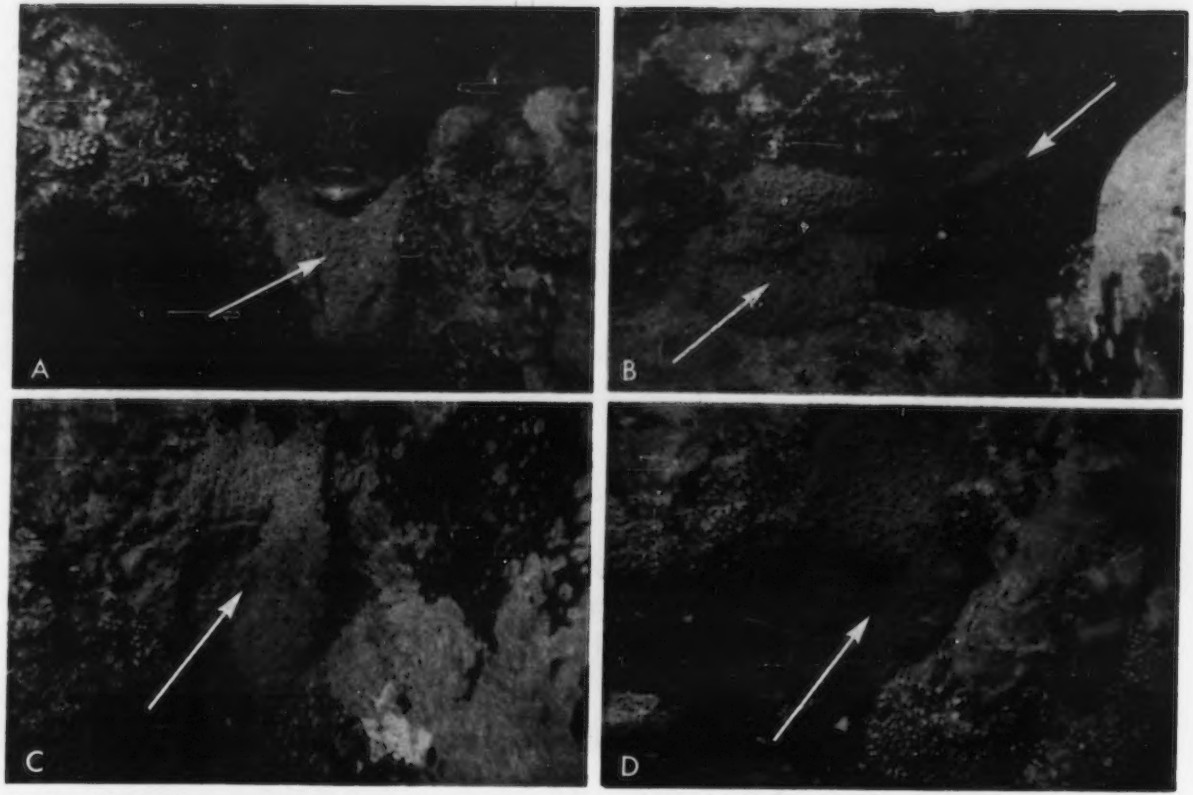

FIG. 2. (A) Shorthorn sculpin guarding an egg mass; (B) shorthorn sculpin guarding two separate egg masses (part of second egg mass visible along the back above the dorsal fins); (C) unguarded egg mass in crevice; (D) unguarded egg mass with the sea star *Asterias vulgaris* advancing towards it.

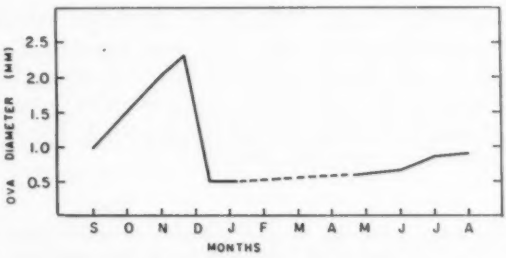

FIG. 3. Seasonal variation in diameter of maturing ova in shorthorn sculpins from Bay Bulls.

participate in the spawning act because each egg mass is guarded by only one male (see section on postspawning behaviour). Also during the spawning period the ratio of mature males to mature females on the spawning grounds is almost 1:1 (Fig. 4). On several occasions one male has been seen guarding

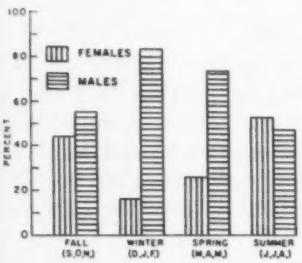

FIG. 4. Seasonal variation in sex ratios in shorthorn sculpins on the spawning grounds in Bay Bulls.

two egg masses. These may be two spawnings from the same female; however, the two egg masses are sometimes of different colours, which suggests that they are spawnings of two females.

SPAWNING SITE

Shorthorn sculpins lay their eggs in individual masses on the bottom, usually in V-shaped crevices between fairly large boulders and in corners of underwater bedrock outcroppings and cliff faces (Fig. 2). These crevices probably aid in the mechanics of spawning and also give better protection from underwater current and surge. Some have been seen shallower than 10 ft (3 m), but most are between 20 and 35 ft (6 and 11 m) and very few are deeper than 50 ft (15 m).

The eggs adhere firmly to each other and the egg mass so formed adheres firmly to the bottom by means of an oviducal secretion (Holt, 1893). The egg masses remain firmly attached even in very shallow water during extremely rough seas.

POSTSPAWNING BEHAVIOUR

Soon after spawning the females move into deep water away from the spawning area. Sex ratios vary in the spawning area seasonally (Fig. 4). Before

spawning began, 59% of the combined samples were females, but after the spawning period, only 10% were females. The immature females apparently move into deeper water just before spawning begins for they were more numerous than the mature females in samples throughout the fall but less in samples during and after the spawning period. Even immature males become scarce after the spawning period.

	Males		Females	
	Immature	Mature	Immature	Mature
Sept. 17, 1966	2	5	6	5
Oct. 22	3	5	8	4
Nov. 19	2	12	7	2
Dec. 3	2	8	1	6
Jan. 21, 1967	0	20	0	4
Feb. 18	1	18	0	0
Mar. 18	0	11	0	1

Although these samples were usually collected in less than 60 ft (18 m) of water, many dives were made to depths of 100 ft (31 m) throughout the winter and very few shorthorn sculpins were seen between 75 and 100 ft (23 and 31 m). Most of those that move off apparently go deeper than 100 ft (31 m). They move back to shallow water again in April–May after the eggs have hatched.

The mature males remain on the grounds to guard the egg masses. The male is usually seen "resting" on the eggs like a bird on a nest (Fig. 2A, B). They have not been observed to clean or aerate the eggs and apparently their main purpose is to protect the eggs from predators, for unprotected egg masses are easily available as food to sea urchins (*Strongylocentrotus dröbachiensis*), sea stars (*Asterias vulgaris*) (Fig. 2D), and spider crabs (*Hyas* sp.). The male sculpin remains with the eggs even during heavy swells when a diver has to hold on to something to remain stationary on the bottom.

Male sculpins are more aggressive when guarding eggs than when they are not. When approached, sculpins guarding eggs raise their dorsal fins until they are nearly completely spread out; the pectoral fins are similarly spread; if touched, the sculpin becomes very rigid with the fins fully spread. If a diver remains near and particularly if he touches the eggs, the sculpin bites at his fingers and hands and sometimes will hold on tenaciously even when shaken. Sculpins not guarding eggs usually swim away quickly when approached, especially if they are touched.

Occasionally egg masses were seen unguarded. One egg mass that was checked several times during the winter was unguarded once but was guarded on previous and subsequent observations. Apparently the males leave the eggs from time to time, probably to feed, but except for these periods the eggs appear to be guarded continuously until they hatch.

Embryogenesis

Embryogenesis of the shorthorn sculpin progresses very slowly in this area (Table 1) taking over 3 months to complete. Although spawning takes place when water temperatures near the spawning sites are around 3–5 C, for most of the period of embryonic development temperatures are near 0 C, and have been recorded as low as −1.5 C.

Table 1. Dates egg samples were taken from the same egg mass at Bay Bulls, water temperatures near the eggs, and observations on embryonic development.

Date	Water temp (C)	Embryonic development
1965		
Dec. 21	1.9	Eggs 2.3–2.5 mm in diameter. Embryos just forming on outside of yolk.
26	1.9	Embryos forming.
31	0.9	Embryos forming.
1966		
Jan. 16	0.5	Embryos partly formed on outside of yolk sacs.
25	0.5	Embryos formed and attached to yolk sacs — about half the size of the yolk.
Feb. 5	0.5	Eye spots clearly visible.
13	0.5	Eye spots enlarging — embryos and yolk sacs about the same size. Chromatophores appearing as tiny specks on head and along sides above stomach.
20	0.0	Embryos slightly larger than yolk sacs. Chromatophores getting larger.
Mar. 1	−0.2	Yolk sacs about half the size of embryos.
12	−0.2	Yolk sacs about ¼ size of embryos and almost inside embryos.
19	0.0	Yolk sacs small and inside embryos.
26	−0.3	Yolk sacs completely inside embryos and chromatophores fully formed.

Hatching Period

Hatching starts around mid-March in this area. Several egg masses were examined on March 19, 1966, during a dive at Bay Bulls and some of them had empty egg capsules, indicating that some hatching had taken place; other egg masses had few or no empty egg capsules. No larvae were seen, but later in the day many larvae hatched from egg samples taken in the dive and held in a plastic bag partly filled with sea water. In a dive on March 26 numerous newly hatched larvae were seen but egg masses examined consisted mainly of unhatched eggs; on April 2 most eggs were empty. On April 9 there was no trace of the marked egg mass or the male sculpin that had been guarding it. Only a small portion of an egg mass, consisting of empty egg capsules, was seen in this dive. Temperatures recorded during the hatching period were generally less than 0 C and as low as −1.5 C.

Smaller planktonic organisms become particularly abundant just before the hatching period and these probably provide food for newly hatched larvae after the yolk has been completely used up.

Larval Behaviour

Newly hatched larvae from eggs collected during a dive were observed in buckets. Upon hatching the larvae swam vigorously toward the surface but after a series of omnidirectional movements gradually sank to the bottom where usually they remained motionless for a short time before swimming vigorously surfaceward again; sometimes they did not reach bottom before swimming upwards. Although movements were usually vertical, they moved horizontally at all levels also.

In nature the larvae were seen at all levels above the spawning area, with the main concentrations being about 2 ft (.6 m) off bottom. Observations indicated swimming and sinking behaviour as was observed for larvae held in buckets. On one occasion many larvae were seen throughout the water column; bottom depth was about 20 ft (6 m) close to shore, where a particularly rough sea was running. No larvae were seen to be carried ashore into the surge of the current.

Fecundity

Female sculpins collected at Freshwater Bay and ranging in length from 20.1 to 50.5 cm, had fecundities ranging from 4205 to 60,976 (Fig. 5). Considerable variation was found in fecundity between fish of the same length, and also with the same age-group.

Age-group	No. eggs	Avg
6	13,014; 17,196	15,105
7	19,838; 28,613	24,225
8	26,700; 39,300	33,000
9	37,280	37,280
10	43,165; 41,073; 47,414	43,884
11	43,060; 38,861	40,960

DISCUSSION

The reproductive processes of the shorthorn sculpin proceed more slowly in Newfoundland waters than in European waters, most likely because of the colder water conditions that prevail in Newfoundland, since temperature is probably the most important of the environmental factors that influence such processes. Also, certain aspects of the reproductive behaviour of the shorthorn appear to be important in increasing the chances of survival of the eggs and larvae.

The three sizes of ova found in maturing ovaries of shorthorn sculpins in Newfoundland waters are interpreted as representing three generations.

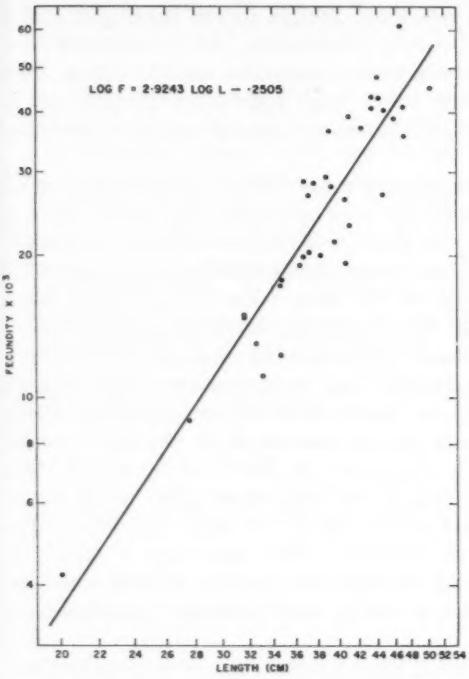

FIG. 5. Length–fecundity relation of shorthorn sculpins from Freshwater Bay.

Lamp (1966) also found three sizes of ova in mature ovaries from shorthorns in European waters but stated that the smallest were newly formed ova of the second generation. He also found that oogenesis took only slightly more than 1 year as compared with 2½ years in Newfoundland waters. This shorter period for oogenesis is related to the earlier maturity (at 2 years) and shorter life span (4–5 years) for European sculpins (Lamp, 1966), compared with an age of 5–7 years for first maturity and life span of 15 years for Newfoundland sculpins (Ennis, 1970). Even with the shorter period of oogenesis for European sculpins some fish spawn only once and few fish spawn more than twice, because mortality increases rapidly after the 2nd year and only a few fish survive 4–5 years (Lamp, 1966).

The spawning period of the shorthorn in European waters is more prolonged than in Newfoundland; it starts at the end of November or the beginning of December (as in Newfoundland) and lasts well into February (Lamp, 1966). The period of embryogenesis lasts only about 5 weeks in European waters (Ehrenbaum, 1905), but in Newfoundland it lasts over 3 months. With the prolonged European spawning period and the short period required for embryogenesis there is considerable overlap in the periods of spawning and hatching; this does not occur in Newfoundland waters. Kändler (1961) reported that the first larvae are taken in January in the Baltic Sea but that hatching

reaches a maximum in February. Shorthorn sculpin larvae have been found as early as February in the Bay of Fundy (Huntsman, 1922) (compared with mid-March for Newfoundland) where winter temperatures are higher than in Newfoundland (Lauzier and Hull, 1962). Such differences are common in many fish species with widespread distributions, and annual variations probably occur in the same area.

The relatively few eggs of the shorthorn are laid in masses attached to the bottom, and embryonic development goes on during the coldest part of the year. During this period the benthic fauna, mainly invertebrates, is relatively inactive because of the low temperatures, and predation on the eggs is at a minimum. At this time also most of the other large bottom-living fishes such as flounders, longhorn sculpins, sea ravens, etc., are in deeper water away from the spawning area of the shorthorn, and those that are present are relatively inactive. The long exposure to relatively light predation apparently is more favourable to survival than a shorter exposure to heavy predation. Being present in fairly shallow water keeps the egg masses clean and well aerated, and predation on the eggs is nearly eliminated by the male's guarding them until they hatch. At any other period of the year these adult males would probably be subject to some predation themselves by other larger fish. The female sculpins become particularly voracious after spawning (Ennis, MS, 1969) and the chances of their eating the eggs and possibly smaller males are eliminated by their moving into deeper water away from the spawning area soon after they spawn.

The larvae hatch during the period when smaller organisms (zooplankton) are particularly abundant and available as food when the yolk sac has been completely used up. The vertical swimming of the larvae probably helps them to maintain position over suitable settling bottom by taking advantage of currents moving in different directions at different layers in the water column. It would also prevent them from being carried ashore by onshore currents. This sort of behaviour has also been suggested for lobster larvae (Squires, 1970).

The most crucial phases of the shorthorn's life cycle occur during the period when physical conditions appear to be most adverse, yet the shorthorn is well adapted to its environment as evidenced by its common and widespread occurrence.

ACKNOWLEDGMENTS

I am grateful to Dr W. Templeman for supervising my graduate work and to Dr H. J. Squires for advice and guidance. Special thanks go to several people who accompanied me on various dives especially to the late Prof H. Lilly (died 1966 in an accident) for his keen interest and enthusiasm to help. Messrs G. E. Tucker, E. L. Rowe, and G. Dawe provided technical assistance in the laboratory and my wife, Mary, spent many hours recording for me as I examined the specimens.

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Temperature Tolerance of Bloater (*Coregonus hoyi*)[1]

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EDSALL, T. A., D. V. ROTTIERS, AND E. H. BROWN. 1970. Temperature tolerance of bloater (*Coregonus hoyi*). J. Fish. Res. Bd. Canada 27: 2047–2052.

Juvenile and young adult bloaters (*Coregonus hoyi*) were tested for tolerance to high temperatures. The ultimate upper lethal temperature of juvenile bloaters (26.75 C) appeared to be slightly higher than that of young adult bloaters, but was similar to that of juvenile ciscoes, *Coregonus artedii* (26.0 C), the only other North American coregonine for which a detailed description of temperature tolerance has been published.

Regression equations are given that permit estimation of the thermal tolerance when the lethal temperatures are incompletely known. The estimated thermal tolerance of juvenile bloaters (617 units) was slightly less than that of the brook trout (*Salvelinus fontinalis*; 625 units), but was higher than that of other Salmonidae.

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INTRODUCTION

THIS PAPER describes the temperature tolerance of the bloater, *Coregonus hoyi*, and is a contribution to a continuing study of the environmental requirements of Great Lakes coregonine fishes. Although some aspects of the biology of these coregonines have been described, only one other experimental study (Edsall and Colby, 1970) has been published on the temperature tolerance of this important group of North American food and forage fishes.

MATERIALS AND METHODS

Age-III experimental fish were reared from eggs taken in Lake Huron 10 miles east of Oscoda, Mich., in February 1967, and age-I fish were from eggs taken in Lake Michigan 4 miles east of Kenosha, Wis., in February 1969. Both groups of eggs were incubated in flowing water at 5–6 C at the Great Lakes Fishery Laboratory. After hatching, bloaters were held at temperatures that varied seasonally from 7 to 10 C; the temperature was about 8 C for the month preceding the start of the temperature tolerance study.

In early February 1970 approximately 100 age-I fish were placed in each of five 30-liter fiberglass acclimation tanks and the temperature in each tank was increased or decreased at the rate of 1–2 C/day until the desired acclimation temperature of 5, 10, 15, 20, or 25 C (±0.1 C) was reached. After the acclimation temperatures were attained, fish at 10–25 C were held 5 additional days and those at 5 C for 11 additional days.

The upper lethal temperatures of the bloater were determined with 270 age-I fish by the method of sudden exposure to an elevated, constant temperature (Brett, 1952) and for 60 age-I and 17 age-III fish by the slow heating method (Cocking, 1959).

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Tests to determine lethal temperature by the constant temperature method were conducted in rectangular 5-liter polyethylene tanks. Bloaters that had been acclimated to one temperature were taken from their acclimation tank and distributed in groups of 10 among the constant temperature experimental tanks. Temperatures in the experimental tanks were selected by preliminary testing to cover a range that would cause rapid, complete mortality in some groups, and slow or incomplete mortality in others. Temperatures were tested at intervals of 0.5 and 1.0 ± 0.1 C within this range. Fish were observed continuously for the first 14 min and thereafter at time intervals that were increased progressively by a multiple of 1.26 (0.1 log min starting with 14.4 min; Tyler, 1966) until 70–100% mortality was reached, or 10,080 min (1 week) had passed. Additional unscheduled observations were made when possible.

Determination of the upper lethal temperature by the slow heating method was conducted in 30-liter acclimation tanks with age-I bloaters acclimated at 8, 20, and 25 C, and in a 760-liter fiberglass tank with age-III fish acclimated at 8 C. Bloaters acclimated at 8 and 20 C were held at those temperatures for 30 days before they were tested, and fish acclimated at 25 C were held at that temperature for 13 days before testing. Temperatures during the slow heating tests were elevated 1–2 C/day to 22 C and thereafter at a rate of 1 ± 0.1 C/day until 100% mortality was reached. Elevation of temperatures began at 8:00 AM; fish were observed at that time, at 4:30 PM, and at least once between 4:30 PM and midnight each day until all were dead.

Fish were considered dead when spontaneous activity ceased and the fish did not respond to strong mechanical stimulation with a glass rod. Preliminary testing showed fish in this condition would not recover when returned to lower temperatures.

All fish that died in the test tanks or that survived for 10,080 min in the constant temperature tanks were measured (total length). The 270 age-I fish used in constant temperature tests averaged 60.0 mm (SD 5.8). The age-I fish acclimated to 8, 20, and 25 C and used in slow heating tests (20, 30, and 10 fish, respectively) had average lengths of 61.3 (SD 5.6), 62.5 (SD 2.9), and 56.8 (SD 7.1), respectively. The 17 age-III fish had an average length of 170.6 mm (SD 12.0). All but one of the age-III fish (3 females, 13 males, and 1 of unknown sex) were sexually mature (capable of spawning in February 1971), and all of the age-I fish were immature.

All fish were exposed to the same photoperiod during acclimation and lethal testing; lights were turned on at 6 AM and were turned off at 9 PM. Each tank was partly covered with a lid, but fish showed no obvious preference for either the shaded or unshaded portion of the tank. A water flow of 0.1, 1.0, and 3.8 liters, respectively, was maintained through each 5-, 30-, and 640-liter acclimation and test tank. Dissolved oxygen remained above 91% saturation in the acclimation and test tanks. The laboratory water supply has a total hardness of 245–355 mg/liter $CaCO_3$ and a *p*H of 7.8–8.1 (Edsall and Colby, 1970). A more detailed description of the chemical characteristics of the laboratory water supply is given by Colby and Brooke (1970).

LETHAL TEMPERATURES

Mortality data for the constant temperature lethal tests were analysed according to Fry et al. (1946). Resistance times of individual fish were plotted on semilog probability paper as accumulated percentage dead and the median resistance time for the test group was determined graphically. The mortality distributions for bloaters were similar to those obtained for ciscoes (Edsall and Colby, 1970) and to those published for most other fishes.

The curves of Fig. 1 show the effect of acclimation temperature on median resistance time and incipient lethal temperatures of the bloater. The "incipient" lethal temperature — the test temperature that kills exactly 50% of the test population during unlimited exposure (Fry et al., 1946) — corresponds to the test temperature at which the curves for fish acclimated at 5–25 C level off. In the present study the incipient lethal temperature was taken as that temperature falling midway between the test temperature that killed 50% or more

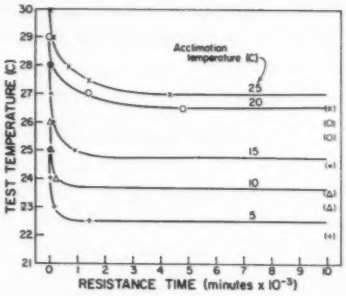

FIG. 1. Median resistance times of age-I bloaters acclimated at various temperatures from 5 to 25 C and tested by the constant temperature method (in parentheses, test groups that had less than 50% mortality in 10,080 min; see text for explanation).

of a test group of 10 fish in 10,080 min and a second test temperature that was 0.5 C closer to the acclimation temperature and killed less than 50% of a second group of bloaters in 10,080 min. For example, the lethal temperature for bloaters acclimated at 15 C was considered to be 24.75 C (Fig. 1), which is midway between 25.0 C (the lowest temperature at which 50% or more of a test group died) and 24.5 C (the temperature at which less than 50% of the test group died after 10,080 min of exposure).

The incipient upper lethal temperature for the bloater varies directly with acclimation temperature up to an acclimation temperature of 22.4 C (Fig. 2) and is described by the equation

$$Y = 21.00 + 0.26\,X \qquad (1)$$

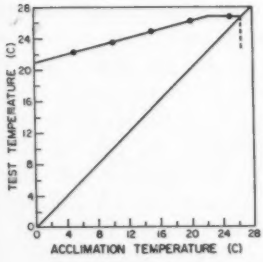

FIG. 2. Upper thermal tolerance of age-I bloaters. Solid circles are upper lethal temperatures determined by the constant temperature method; see text for explanation.

(SE = 0.112, $r = 0.997$; $P < 0.01$, for df = 2) where Y is the acclimation temperature, X is the incipient lethal temperature, 21.0 is the Y-intercept at X equals 0, and 0.26 is the slope, or rate at which the incipient lethal temperature increases for each 1 C increase in acclimation temperature. According to Fig. 2 and equation 1, the incipient upper lethal temperature reached a maximum of 26.75 C at an acclimation temperature of 22.4 C. The maximum incipient upper lethal temperature is also the "ultimate" upper lethal temperature ("the temperature beyond which no increase in lethal temperature results from further increase in acclimation temperature" — Fry et al., 1946). The ultimate upper lethal temperature of age-I bloaters (26.75 C) determined

by the constant temperature method is closely similar to that of age-0 ciscoes (26.0 C) reported by Edsall and Colby (1970).

EFFECTS OF SIZE AND AGE

No relation between fish length and resistance time could be demonstrated for age-I fish tested by the constant temperature method. In tests where all 10 fish of a group died, the slope of the regression of fish length on order of death (a measure of resistance time) was not significantly different from zero ($P > 0.05$). In test groups where mortality was less than 100% the average length of the survivors (57.1 mm; SD 7.9) was slightly greater than that of the fish that died (54.9 mm; SD 6.2), but a *t*-test showed the difference was not significant ($P > 0.05$).

In the slow heating experiments with age-I bloaters, all of the fish acclimated at 25 C died at 28 C, all acclimated at 20 C died at 27–28 C, and those acclimated at 8 C died at 28–29 C; the age-III fish acclimated at 8 C died at 26–27 C. Although the results of the slow heating lethal tests on age-I bloaters varied considerably, the data suggest that the ultimate upper lethal temperature of age-III bloaters may be lower than that of the age-I fish.

The ultimate upper lethal temperatures of adult bloaters larger than the age-III fish used in the present study are unknown, but older, larger ciscoes in nature prefer colder water than smaller younger individuals (Fry, 1937) and the same preference may be true for other species (Ferguson, 1958), including the bloater. If the preferred temperatures are higher among fish that have higher ultimate upper lethal temperatures, as is suggested by the limited data on preferred temperatures and ultimate upper lethal temperatures summarized by Ferguson (1958) and Brett (1956), respectively, then the ultimate upper lethal temperatures in nature may be lower for the older, larger adult bloaters than for the juveniles and young adults. Unfortunately it is not known whether adult and juvenile bloaters in nature have different preferred temperatures. Adult bloaters in Lake Michigan concentrate on the bottom at 8–10 C in late summer (Wells, 1968), but the exact distribution and the preferred temperature of the juveniles are unknown because attempts to capture them during late summer (when they are pelagic) have been mostly unsuccessful. The limited available information suggests, however, that juvenile bloaters concentrate in the area of the thermocline in late summer in water warmer than 8–10 C.

THERMAL TOLERANCE

The thermal tolerance as first described by Fry et al. (1942) is the sum of the tolerance units enclosed within a trapezium formed by the upper and lower temperatures, in which the unit of tolerance is equal to the area bounded by 1 C and expressed as degrees centigrade squared. For the present discussion we found it convenient to divide the tolerance trapezium into upper and lower thermal tolerance zones. The diagonal that bisects the angle formed by the

intersection of the ordinate and abscissa, as in Fig. 2, is the boundary between the upper and lower thermal tolerance zones of the trapezium.

The upper thermal tolerance of bloaters (297 units, Fig. 2) determined by the constant temperature method is in good agreement with the value of 316 units predicted from Fig. 3 and the equation

$$Y = -225.292 + 20.290\ X \qquad (2)$$

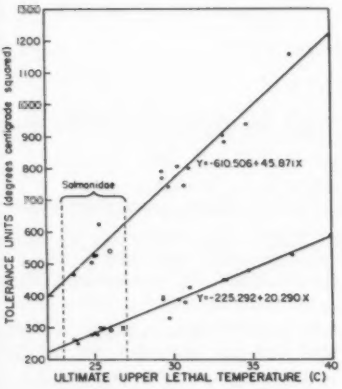

FIG. 3. The relation between ultimate upper lethal temperature and thermal tolerance of various freshwater and anadromous fishes in North America. The upper and lower curves are described by text equations 3 and 2, respectively. Data shown as solid circles were taken from Table 1 of Brett (1956), and include representatives of the families Salmonidae, Percidae, Cyprinidae, Catostomidae, and Ictaluridae. Data marked "provisionary" in Brett's table were omitted from this figure, with the exception of the data for *Oncorhynchus gorbuscha* used in the lower curve and in equation 2. Data for *Coregonus artedii* (open circles) are from Edsall and Colby (1970). The data for *Coregonus hoyi* (×) from the present study were not used in calculation of equation 2.

(SE = 35.813, $r = 0.986$; $P < 0.001$, for df = 15) describing the relation between ultimate upper lethal temperature and upper thermal tolerance for freshwater fishes found in the temperate waters of North America. In equation 2, Y is the upper thermal tolerance, X is the ultimate upper lethal temperature, −225.292 is the Y-intercept at X equals 0, and 20.290 is the slope.

The value of 297 (or 316) upper thermal tolerance units for the bloater is at the lower end of the range of values for freshwater fish in temperate North America. The upper thermal tolerance in Fig. 3 (lower curve) ranges from 251 units for pink salmon, *Oncorhynchus gorbuscha*, to 589 units for the goldfish, *Carassius auratus*. The empirically determined upper thermal tolerance of the bloater (297 units) is slightly higher than the values for most other Salmonidae, but is closely similar to that of the cisco (293 units) and the brook trout, *Salvelinus fontinalis* (300 units; Fig. 3).

Although the lower lethal temperatures of the bloater were not determined in the present study, the thermal tolerance (upper plus lower thermal tolerances) can be obtained from Fig. 3 (upper curve) and the equation

$$Y = -610.506 + 45.871\ X \qquad (3)$$

(SE = 16.578, $r = 0.985$; $P < 0.001$, for df = 16) where Y is the thermal tolerance, X is the ultimate upper lethal temperature, −610.506 is the Y-intercept at X equals 0, and 45.871 is the slope. The thermal tolerance for

the bloater from equation 3, assuming an ultimate upper lethal temperature of 26.75 C, is 617 units and the lower temperature tolerance (obtained by subtracting 297 or 316 from 617) is 301 or 320 units, depending on whether the predicted or empirical value for upper thermal tolerance is used.

Although regression equations 2 and 3 predict the thermal tolerance with a reasonable degree of accuracy, no method has been found that will predict incipient lethal temperatures. Probably, however, the bloater can be acclimated successfully to temperatures approaching 0 C, as can the cisco (Edsall and Colby, 1970) and most other fish native to the temperate fresh waters of North America (Brett, 1960).

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Depth Distributions of Various Stages of Witch Flounder (*Glyptocephalus cynoglossus*) off Nova Scotia and in the Gulf of St. Lawrence

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POWLES, P. M., AND A. C. KOHLER. 1970. Depth distributions of various stages of witch flounder (*Glyptocephalus cynoglossus*) off Nova Scotia and in the Gulf of St. Lawrence. J. Fish. Res. Bd. Canada 27: 2053–2062.

Larvae, juveniles, and adults of witch flounder off Nova Scotia and in the Gulf of St. Lawrence were found to occupy largely discrete niches. The only larvae found (up to 3.8 cm long and presumed to be under 1 year old) were taken at depths of from 0 to 20 fath (0 to 36 m). Fish newly metamorphosed and up to 30 cm long (5 years old) were caught mainly at 100–160 fath (180–288 m). Adults were taken in summer at 20–150 fath (36–270 m) and in winter at 50–240 fath (90–432 m), suggesting that seasonal movements may take place. The centres of abundance of adults were delineated, but not of larvae or juveniles.

It is inferred that the deepwater distribution of young witch flounder prevents direct food competition with young of the more abundant species, cod and American plaice, on the continental shelf areas. Barring unforseen exploitation of the deepwater nursery grounds, witch flounder possess a natural conservation mechanism because of the niche separation between juveniles and adults.

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INTRODUCTION

IN RECENT YEARS witch flounder, or greysole, has become an important commercial species in eastern Canadian fisheries, particularly in the Nova Scotia banks area. However, little is known of its basic ecology. Bigelow and Schroeder (1953, Gulf of Maine), Leim and Scott (1966, Atlantic coast), and Molander (1925, Swedish and Norwegian waters) make no mention of differential distribution of young and adults or of seasonal movements. Bowers (1960), working in the Irish Sea, found that young and adults occurred together in the same depths. However, that witch flounder ecology and habitat off the Canadian east coast differ considerably from those in European waters became obvious when attempts were made to capture young fish. This study provides evidence for ecological isolation between the larvae, juveniles, and adults, off Nova Scotia and in the Gulf of St. Lawrence, and suggests that short but regular seasonal movements are undergone by the adults. These aspects have not been previously documented for the species.

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METHODS

The depths at which the various stages occur were determined from research surveys and samples from commercial catches. In the research surveys, the following gears were used: standard #41.5 Iceland trawl with small mesh liner and #36 Yankee otter trawl and liner (adults and juveniles); 12-ft Isaacs-Kidd midwater trawl and 1-m plankton net (larvae and juveniles); and Norwegian deep-sea shrimp trawl (see Squires, 1961, mainly juveniles).

We measured lengths from the tip of the snout to the end of the longest caudal ray, and recorded the sex. Saccular otoliths provided a measure of age using the method described by Powles and Kennedy (1967).

Squires (1961) made available to us his field data on catches of juvenile witch flounder taken during shrimp surveys.

DEPTHS OF CAPTURE OF VARIOUS SIZES

In the research surveys in both areas studied, the adults, juveniles up to 30 cm long, and larvae were taken largely at discrete depths.

Adults

Four summer (May–October) and three winter (November–April) research cruises were made in each of the study areas to ascertain the depth distribution of adults. The greatest numbers were taken at 51–90 fath (92–162 m) in summer and at 121–180 fath (218–325 m) in winter (Fig. 1). The mean numbers per

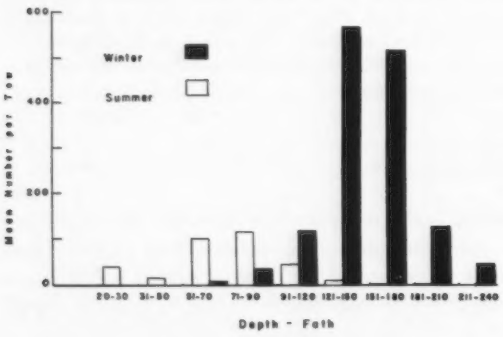

FIG. 1. Depth distributions of adult witch flounder in research catches from the Nova Scotia banks area during two seasons (winter: November–April 1964–65; summer: May–October 1965–66).

tow also indicated that the fish were more concentrated in winter than in summer months, a phenomenon that has often been reported by fishermen.

Juveniles

Juveniles under 30 cm long were never observed at processing plants, where some fish of precommercial size are discarded for most species. This suggested that only commercially acceptable sizes were caught at sea, a suggestion that was supported by direct observation (Fig. 2) of catches on the Nova Scotia banks at 60–80 fath (108–144 m) by two commercial vessels. Few witch flounder under 30 cm were captured by 4.5-inch (11.4-cm) mesh codends, even though the 50% retention point for such a net is a length of 25 cm.

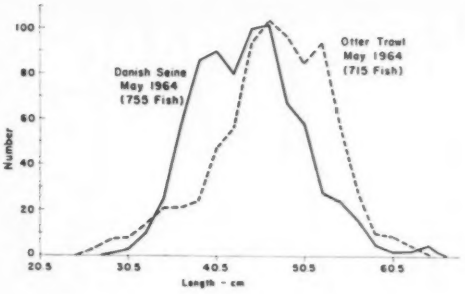

FIG. 2. Size distributions of commercial catches of witch flounder by otter trawl on Nova Scotia banks at 60–80 fath (100–140 m), with 4.5-inch (11.4-cm) mesh codend.

Several research cruises were made on the Nova Scotia banks with otter trawls covering depths of from 20 to 250 fath (36 to 450 m) for comparison with the commercial catch records. Even with 1.5-inch (3.8-cm) liners, precommercial witch flounder were captured at these depths only in insignificant numbers (Fig. 3).

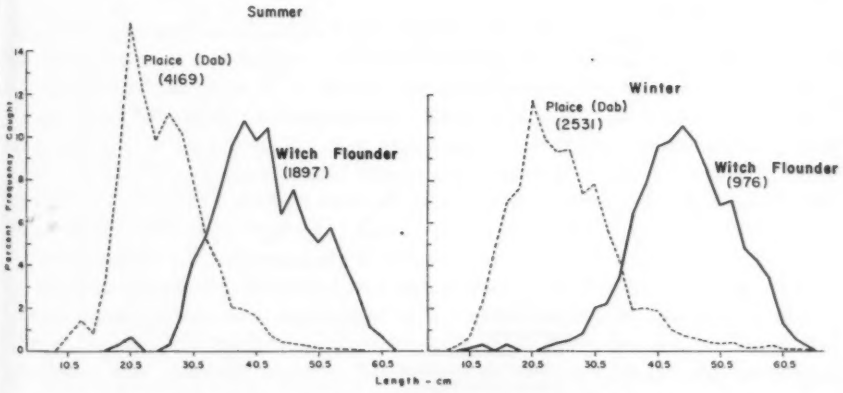

FIG. 3. Size distributions of research catches of witch flounder and American plaice by otter trawl on Nova Scotia banks in summer and winter at 20–250 fath (36–450 m) with 1.5-inch (3.8-cm) mesh codend, 1963.

Of the several possibilities that could account for lack of young in the catches, escapement through wings of nets was ruled out because many small American plaice were captured on the same grounds (Fig. 3); unless American plaice and witch flounder differ radically in behaviour, one would have expected to take witch flounder also if they were present.

Attempts made with a deep-sea shrimp trawl to locate nursery grounds on the Nova Scotia banks were moderately successful (Fig. 4), although hauls at great depths and over rocky bottom were difficult with such light gear. Fish under 30 cm (2–5 years old) formed the bulk of the catches, and were taken in water primarily over 100 fath in depth. Squires' (1961) data also

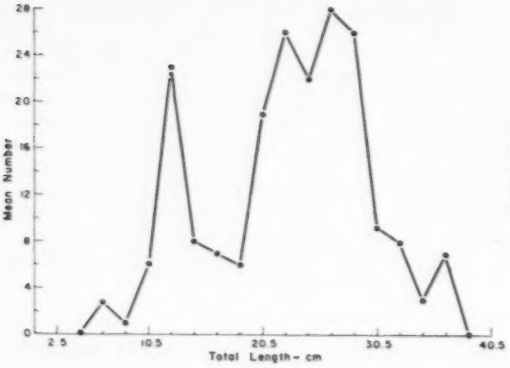

FIG. 4. Size distributions of research catches of juvenile witch flounder on Nova Scotia banks with a deep-sea shrimp trawl, April–November 1965. Depths trawled, 60–150 fath (100–270 m); mesh size 1.5 inches (3.8 cm).

indicate (Fig. 5, insert) that juveniles were taken from depths over 100 fath, with peaks at 100 and 140 fath.

LARVAE

Fishing with an Isaacs-Kidd midwater trawl yielded less than a dozen small larvae, all in the 3-cm range. These were taken by oblique tow in water from 40 to 100 fath (72 to 180 m) deep. Kelly and Marak, U.S. Bureau of Commercial Fisheries, Woods Hole, Mass. (personal communication), captured large numbers of witch flounder up to 6.8 cm in length with the same gear in the western Gulf of Maine. Their fish were taken above the thermocline, at 16–22 fath (30–40 m) from the surface, but at depths over 80 fath (144 m). The larger individuals were very close to metamorphosis (and they probably would have moved to the bottom in a very short time). This suggested that our failure to capture many larvae in Nova Scotia waters was a result of fishing wrong depths, not an indication of their absence. This hypothesis was strengthened when fish taken by shrimp trawl (Fig. 4) measured more than 6.5 cm in length, suggesting that most remained pelagic until at least this size was reached.

DISTRIBUTION AND MOVEMENTS OF ADULTS

The geographical distributions of adult witch flounder in the study areas were not documented in detail. In winter, adults were caught off Nova Scotia by both research and commercial vessels only in deep holes on the banks, the main stocks evidently migrating into the deeper water (Fig. 1). Adults were taken widely off Nova Scotia and in the Gulf of St. Lawrence by both research and commercial vessels in both summer and winter (Fig. 6, 7). The distances moved may be so short that, when commercial log records are plotted, they do not bring out the movements. A stock of witch flounder may only move 5–10 miles before reaching their winter preferendum. Movements of a longer nature do also occur, however. In one area (1 in Fig. 6), which is located southeast of Cape Breton Island, the stock occurred in the summer months in shallow

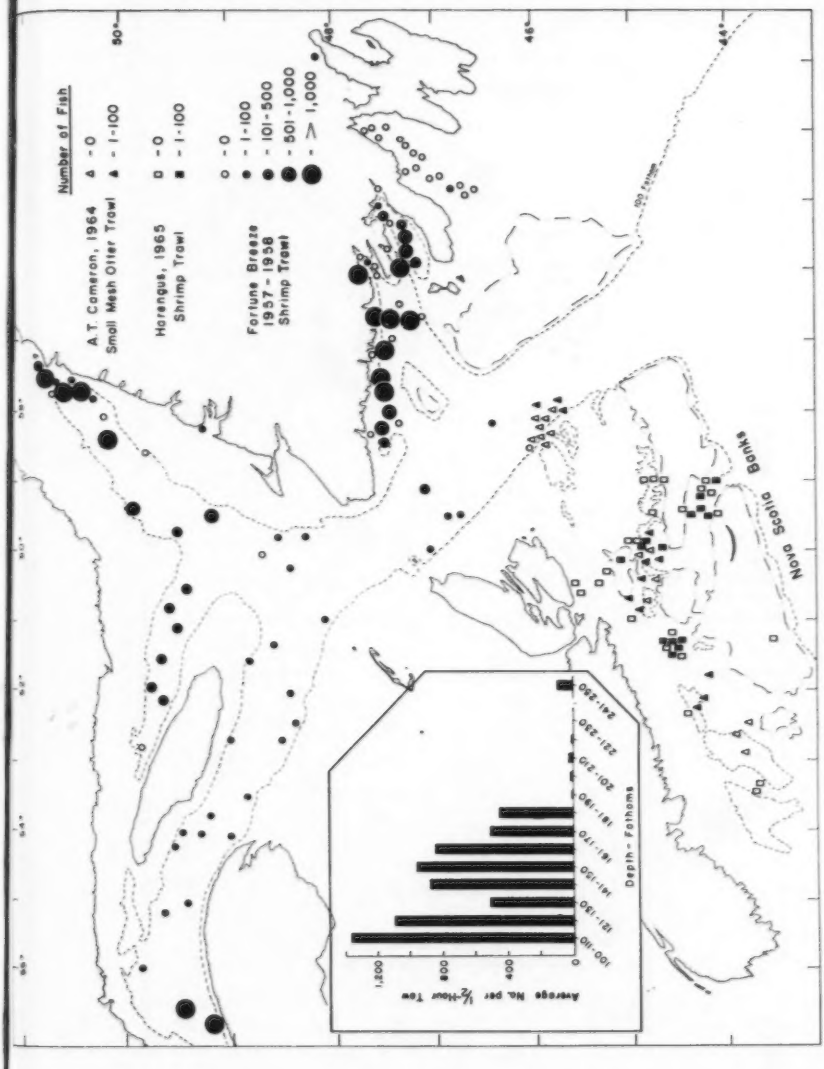

FIG. 5. Geographic distributions of research catches (numbers per ½-hr tow) of juvenile witch flounder by otter and deep-sea shrimp trawls off eastern Nova Scotia, summer and winter 1965–67, and (field data of Squires, 1961) by deep-sea shrimp trawl in Gulf of St Lawrence. Inset, depth distribution of catches of juveniles in Gulf in summer, 1957–58 (Squires' data); few tows were made at depths below 100 fath (180 m).

waters, but in the winter months was found to the southeast in deep water along and over the 100-fath contour.

In the Gulf of St. Lawrence, adults occurred widely inside the 100-fath contour in summer, but in winter they tended to move to deep water along the 100-fath contour and the west coast of Cape Breton. The same held true for the Nova Scotia banks fish except that there are an appreciable number

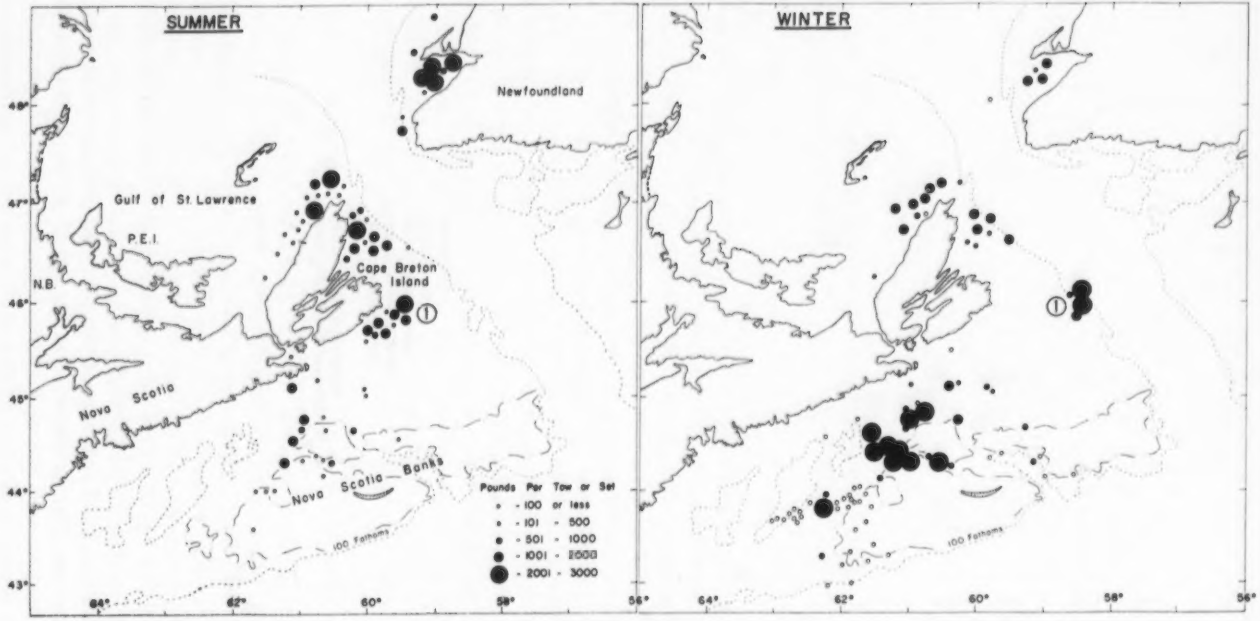

FIG. 6. Geographical distributions of catches of witch flounder by commercial vessels off Nova Scotia and in Gulf of St. Lawrence in summer and winter, 1961–62.

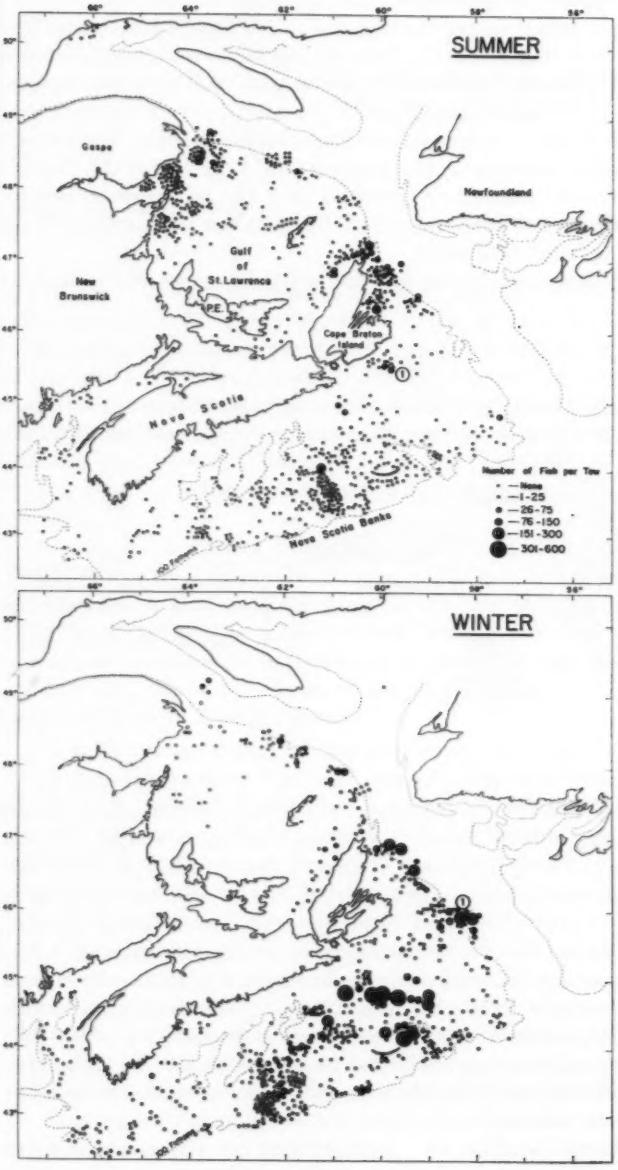

FIG. 7. Geographical distributions of catches of adult witch flounder by research vessel off Nova Scotia and in Gulf of St. Lawrence in summer and winter, 1961–67.

of deep holes right on the banks, where adults often remain throughout the winter and which cannot be indicated on small-scale charts.

Juvenile fish were found widely in both the Nova Scotia banks area and the Gulf of St. Lawrence (Fig. 5). In the Nova Scotia banks area small fish were found mainly in deep holes, but areas off the continental shelf were not explored to any great extent. The most comprehensive picture of juvenile distribution in the Gulf and Cabot Strait was obtained from Squires' (1961) field records collected during shrimp surveys in the summers of 1957 and 1958. Squires (Fig. 5) took the greatest number of juveniles near the midwestern and the southwestern coasts of Newfoundland, fewer being taken off Gaspé and not many near the Magdalen Shallows.

Geographic distribution of larvae was not plotted because of the scarcity of data.

DISCUSSION

Witch flounder life history is unusual and complicated because the animal clearly occupies at least three largely discrete ecological niches (Fig. 8).

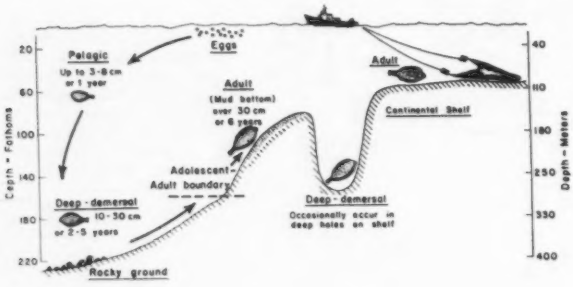

FIG. 8. Diagrammatic representation of niche separation in witch flounder in study area. "Deep-demersal" refers to juveniles up to 30 cm.

The egg hatches into a pelagic larval stage, which may persist up to a year (Bigelow and Schroeder, 1953). Larvae are difficult to capture, and the only ones caught by us were less than 38 mm in length. The evidence of Kelly and Marak (personal communication) suggests that the larvae are sometimes concentrated at 16–22 fath (30–40 m) from the surface over deep water.

The next phase occurs in very deep water when metamorphosed fish settle on bottom. In most species of flatfish the juvenile stages that follow the pelagic larvae are found in water no deeper than that in which the adults are taken. The witch flounder is unusual in that juveniles up to 30 cm (2–5 years old), those in the deep-demersal phase, are found in depths of 80–250 fath (144–450 m), mainly at 100–160 fath (180–288 m), where they are discrete from the adult stock except in winter.

Factors determining duration of the deep-demersal phase are not yet known. Certainly it is not the maturation process. Examinations of length and age at maturity for witch flounder (Fig. 9) show that males mature at

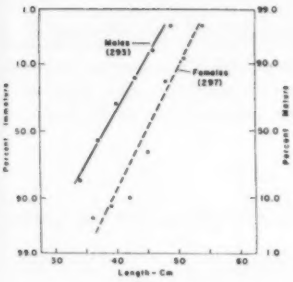

FIG. 9. Percentages of male and female witch flounder taken by otter trawl in research survey on Nova Scotia banks that were mature at various lengths, May 1965.

37 cm (age 7 years) and females at 44 cm (age 10 years). Even if only 5% of males matured at 30 cm (as suggested by Fig. 9), and only mature fish migrated to the banks, we would expect to find significant numbers of such fish in catches on the banks. However, all fish below 30 cm were actually juveniles and were taken in only very small numbers. There might exist a late-juvenile or adolescent stage similar to that exhibited by adolescent Barents Sea cod, which make "dummy runs" for spawning, as noted by Woodhead in Baggerman (1960). During these movements they joined the adult stock before actual maturity set in.

There could also be other factors involved. For example, very little is known of the feeding habits of these small fish, but possibly with increased size they follow food resource changes, and the larger of the small fish migrate to shallower depths for different food. The stimulus for initiating shoreward movement could be the intercontact of juveniles with the periphery of adult stocks during the winter. Towards spring, as adults move into shallower water, some adolescents would join them.

The significant aspect of this life history for fisheries management is that witch flounder have a "built-in" conservation mechanism for the first 4–5 years of life. While young witch flounder are pelagic or in deepwater areas, the only fishery that might threaten or harm them is that for redfish (*Sebastes marinus*). Shrimp are not yet fished commercially in the nursery areas. The rough ground and great depths at which the young occur are current safeguards against depletion by present trawling operations.

There are still great gaps in the knowledge of the ecology of this species. It is not known yet whether most witch flounder spawn in deep or in shallow water. Since the breeding season appears to be protracted (Bigelow and Schroeder, 1953), it is likely that spawning occurs at depths of 140–40 fath (260–70 m) depending on the time of the year. This suggests that a fairly complex type of behaviour is required in the pelagic larval phase in seeking the deep waters.

The deepwater phase of the small witch flounder precludes direct feeding competition on the main fishing grounds with small American plaice (*Hippoglossoides platessoides*) and Atlantic cod (*Gadus morhua*), the most abundant groundfishes in the area.

ACKNOWLEDGMENTS

The authors thank Drs D. Carlisle and J. S. Scott and Mr S. N. Tibbo for criticism of the dratf manuscript; and Drs H. J. Squires, G. Kelly, and R. Marak, U.S. Bureau of Commercial Fisheries, Woods Hole, Mass., for supplying field data on catches of juvenile witch flounder. Field work for the investigation was carried out by the staff of Groundfish Investigations at St. Andrews, and research vessel personnel.

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Growth of Cutthroat Trout (*Salmo clarki*) in Chef Creek, Vancouver Island, British Columbia[1]

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COOPER, E. L. 1970. Growth of cutthroat trout (*Salmo clarki*) in Chef Creek, Vancouver Island, British Columbia. J. Fish. Res. Bd. Canada 27: 2063–2070.

Cutthroat trout were collected periodically from April 3 to September 20, 1968, by electrofishing and were preserved in formalin. Within a few days, each fish was measured and a sample of scales was taken for study. Scales first appeared on these cutthroat trout immediately above and below the lateral line on the anterior portion of the caudal peduncle. Scales were always selected from this area for study, mounted in glycerin–gelatin, and examined at a magnification of 127 diameters. Scales grow relatively more slowly as the fish increases in length, causing the body–scale relation to be curvilinear. Growth of the fish was determined both by calculating size-at-age data from the scales and by comparing mean lengths of age-groups collected at different times of the year.

The growth of cutthroat trout from Chef Creek was slow; calculated mean fork lengths for the first three annuli were 50, 84, and 119 mm. Only a few fish of age-group IV were collected and these were considered to be nonanadromous individuals on the basis of scale examination and small size.

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INTRODUCTION

MANY STREAMS draining the coastal area of northwestern North America contain mixed salmonid populations of fish. The cutthroat trout, *Salmo clarki*, frequently is a component of these populations and may be represented by a mixture of both anadromous and resident forms. Such is the case in Chef Creek, where both forms are known to spawn.

Chef Creek is less than 8 km in length and averages 3–4 m in width, draining from the eastern slope of Vancouver Island about 80 km north of Nanaimo. The gradient is moderate, the watershed is almost entirely wooded, and the bed of the stream is predominantly gravel and rubble. The flow varies seasonally, but minimum summer flow remains above 30 liters/sec. The maximum water temperature recorded during the summer of 1968 was 14 C, but it rarely was higher than 12 C.

The fish population is dominated by cutthroat trout and coho salmon, *Oncorhynchus kisutch*. Cutthroat trout are found throughout the length of the stream whereas coho are chiefly restricted to the lower two-thirds of the stream. Because of a very heavy migration of adult coho into the lower part

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of the stream, the resultant population of young coho is several times as great as the combined population of anadromous and resident cutthroat trout. Studies on competition between young coho and cutthroat trout are underway in the stream by other investigators. The present study established the growth rate of the cutthroat trout population based on a detailed analysis of scales from this species.

METHODS

Trout were collected periodically from April 3 to September 20, 1968, with a pulsed d-c electrofishing apparatus and immediately were preserved in 10% formalin. Within a few days, each fish was measured (fork length (FL) in millimeters), weighed, and numbered for future reference.

Scales were removed from each of 850 specimens from an area between the posterior margin of the dorsal fin and the anus, limited to the first three scale rows either above or below the lateral line on the right side of the fish. This area was selected to coincide with the area where the first scales were shown to appear on other salmonids. From the 50–100 scales removed from each fish, three were selected as being most uniform in size and shape, being particularly careful to reject all scales showing any degree of regeneration. These three scales were then mounted in glycerin–gelatin for detailed analysis.

The trout scales used to calculate size-at-age data for this study were mounted in glycerin–gelatin. Since Berleze's fluid is also a common mounting medium used by microscopists and has many desirable features, a comparable set of scales were mounted with this medium. These samples were selected in a similar manner to those mounted in glycerin–gelatin but were taken from the left side of the fish. When scales mounted in Berleze's fluid were examined, it was noted that considerable shrinkage of the scales had occurred within a few days after mounting (Table 1).

TABLE 1. Measurements of anterior scale radius of cutthroat trout scales mounted in Berleze's fluid compared with measurements from scales mounted in glycerin–gelatin. Number of individual fish in parentheses, three scales from each fish (ASR values in micrometer units; each unit equals 12.7 μ).

Size group of trout (*FL in mm*)	Mean ASR of scales	
	in Berleze's fluid	in glycerin–gelatin
40–49	14 (38)	16 (39)
50–59	12 (162)	19 (162)
60–69	19 (120)	22 (120)
70–79	21 (87)	26 (87)
80–89	23 (36)	30 (36)
90–99	26 (33)	32 (33)
100–109	27 (72)	34 (72)
110–119	29 (42)	37 (42)
120–129	31 (18)	41 (18)
130–139	30 (15)	39 (15)
140–149	37 (6)	48 (6)
150–159	34 (12)	44 (12)
160–169	33 (3)	43 (3)

This shrinkage also changed the relative spacing of circuli on the scales, and it was very difficult to interpret the age of the fish. The scales mounted in Berleze's fluid were then discarded as unusable.

RESULTS

Size at Scale Formation

Scales first form in cutthroat trout on the region of the body described above when the fish reach a fork length of approximately 36 mm (Table 2).

TABLE 2. Fork length at which scales first appear in cutthroat trout. Area sampled is immediately anterior to the anus and two to three scale rows above and below the lateral line.

FL (*mm*)	31	32	33	34	35	36	37	38	39	40	41	42	43	44
No. fish without scales	10	10	10	11	5	3	0	0	0	0	0	0	0	0
No. fish with scales	0	0	0	0	0	5	7	10	10	10	10	11	9	10

No fish less than 36 mm had scales, and all fish longer than 37 mm were scaled on the sampling area. The smallest scale platelets observed had an anterior radius of about 0.1 mm and no circuli were then apparent. These initial platelets grew considerably in size before the first circulus appeared at some distance from the margin of the scale.

Rate of Regeneration With Age

Much of the difficulty in working with trout scales arises from their high rate of regeneration as the fish gets older. To document this, about 10 fish were selected from each centimeter size range and the numbers of good scales and regenerated scales in each sample were counted (Table 3). The rate of regeneration increased from about 40% for yearling fish 40–49 mm FL to more than 80% for fish larger than 140 mm. A cutthroat trout from Chef Creek would be about 140 mm during its 3rd year of life. The loss of scales and their subsequent regeneration appears to be a function of time, for young-of-the-year trout 40–49 mm in length, sampled in July, had a scale regeneration rate of only 3%, compared with 40% for yearling fish of the same size.

Relation Between Fork Length and Anterior Scale Radius

For a preliminary analysis, the mean anterior scale radius (ASR) for different size ranges were plotted against fork length of the fish (Fig. 1). Inspection of the distribution of these mean values, based on three scales from each of 850 fish, suggested that the data could be fitted arbitrarily by three straight lines with inflection points at fork lengths of 45 and 120 mm. Since these inflection points have no discernible relation to the biology of the fish, I attempted a curvilinear fit to the data. The following logarithmic transformations of the data were made to obtain a straight-line fit: ASR:log length; log ASR:log length; and log ASR:length. None was judged satisfactory.

Consequently, a generalized computer program was employed to obtain a fit to the data, with the following solution: $ASR = -5.62 + 0.499L - 0.001 L^2$. This line is drawn on Fig. 1, and appears to be an excellent fit except for

TABLE 3. Number of regenerated scales in relation to size of cutthroat trout. First number in each pair refers to nonregenerated scales, second number refers to regenerated scales in each sample.

FL (*mm*) Age-group	40–49 0	40–49 I	50–59	60–69	70–79	80–89	90–99	100–109
	25-0	23-10	29-36	33-25	15-22	31-29	12-57	7-68
	15-0	15-13	22-23	39-25	15-43	22-31	32-19	17-83
	21-0	35-21	25-30	32-32	34-46	19-44	36-50	17-39
	19-0	9-21	25-29	24-30	11-35	23-32	17-49	47-27
	18-4	34-11	16-22	15-41	28-29	19-32	8-52	31-73
	19-0	33-7	27-5	26-34	17-27	58-47	35-33	27-27
	27-0	18-9	44-8	19-30	48-13	38-20	56-4	18-44
			27-18	29-22	27-34	15-61	23-42	21-52
			18-20	44-10	16-30	22-54	59-26	21-36
			21-29	24-9	27-2	38-51	57-29	11-122
Regenerated (%)		38	45	47	53	58	53	70
FL (*mm*)	110–119	120–129	130–139	140–149	150–159	160–169	170–179	180–189
	14-134	12-59	11-40	8-85	15-67	14-81	14-88	12-114
	19-39	14-50	30-71	21-72	6-93	9-80	26-63	18-48
	17-48	30-41	39-62	11-74	6-223			
	21-53	19-97	11-57	35-91	8-74			
	23-27	40-47	5-655	15-68	54-124			
	23-42	33-33	4-184	6-63	18-96			
	24-74	23-82	24-62	61-73	38-99			
	31-77	38-62	37-47	25-82	36-85			
	74-51	28-69	25-54	11-79	14-58			
	23-64	9-99	18-110	9-200				
Regenerated (%)	69	71	77	82	82	88	79	82

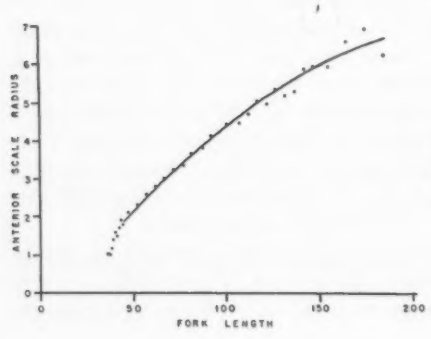

FIG. 1. Anterior scale radius (in decimillimeters) of the cutthroat trout related to fork length of the fish (in millimeters).

fish less than 50 mm in length. A nomograph was then constructed from the computer solution for fish ranging from 50 to 200 mm; empirical values were used for fish less than 50 mm. The nomograph was then used for back-calculating size-at-age data.

In view of the large sample available, and the difficulty of fitting any mathematical expression to these data, Segerstråle's (1933) method of deriving an empirical table from the data would probably have been equally useful in back-calculating size-at-age data.

Growth Calculated From Scales

The growth rate of this population is very slow; calculated mean fork lengths for the first 3 years of life were 50, 84, and 119 mm, based on a rather large sample from this population (Table 4). The four fish in age-group IV

Table 4. Fork length (in millimeters) of cutthroat trout calculated from scale measurements.

Age-group	No. individuals	Size at completed year of life			
		1	2	3	4
I	510	49.7			
II	196	48.9	83.8		
III	32	49.2	84.5	118.9	
IV	4	44.8	81.2	109.0	133.5

(fifth summer) had grown more slowly than the rest, but the sample is too small for meaningful comparisons.

Trout captured in the lower half of the stream drainage grew somewhat faster than those taken from the upper portion. This difference in growth held true for all monthly subsamples collected (Table 5). Only the sample of age-group I fish was large enough to be included in this comparison.

Table 5. Calculated fork length (in millimeters) of cutthroat trout from different portions of Chef Creek. Size at first annulus calculated from scale measurements of samples of age-group I collected in different months; sample size in parentheses.

Portion of stream	Calculated FL at 1st annulus from sample					
	Apr.	May	June	July	Aug.	Sept.
Upper	48.0(63)	45.0(75)	–	48.1(85)	47.3(46)	49.4(47)
Lower	57.0(61)	47.9(15)	–	52.5(46)	51.9(50)	53.0(22)

The analysis of growth of this cutthroat trout population is confounded somewhat by the inability to distinguish resident fish from anadromous individuals. During the sampling period (April–September) no large anadromous

adults were captured or observed in the stream, but many sexually mature resident fish of both sexes were collected.

Empirical Growth From April to September

The seasonal growth history of age-groups 0 and I, constructed from the large monthly samples taken from April to September (Table 6) is consistent with the mean lengths calculated from scales, with one exception. At the end

Table 6. Growth of young-of-the-year (age-group 0) and yearling (age-group I) cutthroat trout in Chef Creek during April through September 1968. Sample size in parentheses.

Age-group and stream section	Mean FL (*mm*) of fish captured in					
	Apr.	May	June	July	Aug.	Sept.
0 Upper	–	25.8(26)	–	35.0(273)	44.2(209)	46.2(135)
Lower	27.2(22)	33.3(53)	–	37.8(207)	47.4(150)	50.7(125)
I Upper	61.2(63)	71.7(75)	–	82.7(85)	86.0(46)	89.5(47)
Lower	59.1(61)	73.2(15)	–	87.8(46)	91.3(50)	92.4(22)

of their second growing season, yearling fish captured in September (presumably before completing their second growing season) were consistently larger than the length calculated from scales at the completion of the second annulus (Table 4, 6). The most logical explanation for this is the possibility that some of the larger individuals of the yearling group move out of the stream into the estuary during late fall or early winter and are therefore not available for capture in the stream during April to September of their third growing season. No further information is available to test this hypothesis.

Most of the yearly increment of growth (about 90%) occurs during April through September, although there is evidence that fish in age-group I start growing earlier in the season than do the young-of-the-year fish. And, as was the case with the calculated length data, cutthroat trout living in the downstream part of Chef Creek grew somewhat faster than did fish living in the upstream portion (Table 6).

DISCUSSION

Scales on cutthroat trout from Chef Creek first appeared in one or two rows adjacent to the lateral line immediately anterior to the anus, as reported previously by Neave (1936) and Brown and Bailey (1952). The precise selection of this small area for sampling (less than 3% of scaled surface) is advantageous for several reasons. The scales are larger and thus easier to work with. The difficulties in correctly assigning age to slowly growing individuals, described in detail by Laakso and Cope (1956), are kept to a minimum by sampling scales from those that appear first in development. Variability in scale measurements is high among fish of the same size even when the sampling area is restrict-

ed; when this precaution is not taken it is very difficult to obtain mean values with an acceptable degree of precision.

A combination of small samples and too large a sampling area has been responsible for many misinterpretations of the relation between scale size and body size. Attempts to depict the body–scale relation of the cutthroat trout as linear (Laakso, 1956; Platts, 1958) were faulty largely on a basis of excessive variability of inadequate data.

Most investigators have reported the relation between scale size and body size for cutthroat trout as being curvilinear (Fleener, 1952; Irving, 1955; Laakso and Cope, 1956; Vernon and McMynn, 1957), although there is not general agreement on what mathematical expression, if any, provides the best fit to the data. Although the lack of standardization of measurements of both scales and fish confounds any comparison, the plotted data of both Fleener (1952) and Irving (1955) appear on inspection to be very similar to our data from Chef Creek. This is especially interesting in view of the widely disparate subspecies of cutthroats that are involved.

Martin (1949) proposed that the relative growth of body parts is characterized by a series of relative-growth stanzas that differ in slope and in the size range over which each is applicable. Our data on scale growth and body growth of the cutthroat trout may be so interpreted with inflections in scale growth occurring at fork lengths of approximately 45 and 85 mm. Smith (1955) reported a similar sharp inflection for scale growth in Kamloops trout (*Salmo gairdneri kamloops*) at 45 mm FL. More information is needed on the developmental biology of these trouts to clarify this interpretation of relative-growth phenomena.

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Behavior of Skipjack Tuna, *Katsuwonus pelamis*, as Determined by Tracking with Ultrasonic Devices

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YUEN, H. S. H. 1970. Behavior of skipjack tuna, *Katsuwonus pelamis*, as determined by tracking with ultrasonic devices. J. Fish. Res. Bd. Canada 27: 2071–2079.

A 50-kHz transmitter was placed in the stomach of a 44-cm (estimated) skipjack tuna, *Katsuwonus pelamis*, at Kaula Bank, Hawaii. With a few interruptions the fish was tracked from August 30 to September 6, 1969. The fish made nightly journeys of 25–106 km away from the bank and, with one exception, returned to the bank every morning to remain there for the day. Another skipjack tuna, about 40 cm long, was tracked for 12 hr at Penguin Bank, Hawaii. It also stayed at the bank during the day and left at night. Both fish swam close to the surface at night but swam at various depths during the day. The repeated morning returns of the skipjack tuna to Kaula Bank imply that skipjack tuna can navigate and that they have a sense of time.

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THE FOREMOST POSITION of the fishery for skipjack tuna, *Katsuwonus pelamis*, in Hawaii is largely maintained by an annual influx of "season" fish of 60–70 cm length (Uchida, 1967). As important as this migratory component is to the fishery, its movements into, within, and out of the Hawaiian area remain a mystery. Shippen (1961), after an analysis of the distribution of skipjack tuna catches in time and space, suggested that the fish entered the fishery from the west and departed towards the west. On the other hand, a method for predicting the annual catch of skipjack tuna of the Hawaiian fishery can be explained only by hypothesizing entry from the northeast (E. C. Jones, personal communication). The capture in Hawaii of two skipjack tuna tagged off Baja California (Rothschild, 1965) gave some support to this hypothesis. A study of movements within the fishery (Royce and Otsu, 1955) through the analysis of data on sightings and visual tracking of surface schools was inconclusive because of the effects of weather conditions on sightings and the brevity of tracking attempts, which did not exceed 15 min.

Smaller skipjack tuna about 40–50 cm long, although they too are seasonal, are caught in small quantities throughout the year. Recoveries of tagged skipjack tuna of this size revealed only localized movements with no obvious pattern (Yamashita and Waldron, 1959). An association of these fish with banks is indicated by the common practice of fishermen to go to areas with banks to fish them. The relation between fish and bank is unknown.

The increasing success at tracking fish bearing ultrasonic transmitters (Trefethen, 1956; Trefethen et al., 1957; Johnson, 1960; Novotny and Esterberg, 1962; Bass and Rascovich, 1965; Hasler et al., 1969; Henderson et al., 1966;

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Dizon et al., 1970) offered an approach for studying the movements of the skipjack tuna. In August 1969, the BCF (Bureau of Commercial Fisheries) Biological Laboratory, Honolulu (now the National Marine Fisheries Service), initiated field work to determine the feasibility of tracking the large commercially important skipjack tuna equipped with ultrasonic transmitters for extended periods and to obtain information on the behavior of the smaller skipjack tuna associated with banks.

As large skipjack tuna were not available at the time, work was limited to smaller fish. From August 30 to September 9, 1969, ultrasonic transmitters were placed in two skipjack tuna and tracked. Methods and results are described and discussed in this report.

MATERIALS AND METHODS

Transmitter characteristics are listed below. The transmitters contained a magnetic reed switch and were activated by the removal of a magnet taped on the outside (Fig. 1).

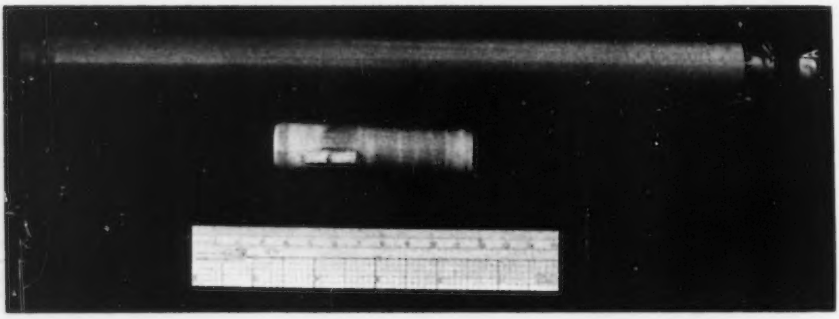

FIG. 1. Tag and applicator.

Frequency	50 ± 1 kHz	Shape	Cylindrical capsule with rounded ends
Repetition rate	2 pulses per sec		
Pulse length	50 msec	Dimensions	8.1 cm long, 2.0 cm diam
Power output	52 db relative to 1 microbar at 1 yard	Weight in air	62 g
		Lifespan	250 hr

Prior to the field work, trial runs were made on captive fish to test a tagging technique and to determine the effects of the transmitter on skipjack tuna. The trials were made on two skipjack tuna 48 cm long. These fish were part of a school that was held in a plastic swimming pool with a capacity of 45 m^3. The transmitters were placed in the stomachs of the fish. The two skipjack tagged in this manner behaved as the other members of the school held in captivity and were not distinguishable from them. Both tuna, one dissected 3 days and the other 4 days after the insertion of the transmitters, contained the remains of food fish in their stomachs, indicating that they had resumed feeding.

Skipjack tuna to be tracked were caught by trolling lures with barbless hooks. As soon as a lure was struck the ship was reversed and the line pulled in by hand evenly and firmly. When the fish was alongside the ship it was scooped up in a dipnet. The hook was removed and the fish tagged while still in the net. Tagging consisted of inserting the transmitter into the pharynx

with a plastic applicator (Fig. 1) and allowing the fish to swallow the transmitter. The fish was released by lowering the net into the water.

Neither of the two fish tracked was measured. The length of the first fish was estimated by taking the mean length of 11 fish that were caught from the same school. The size of the second skipjack tuna was gauged by eye.

Signals from a tag were received through the listening mode of the continuous-transmission, frequency-modulated sonar (Yuen, 1968) on the research vessel *Townsend Cromwell*. The hydrophone, located 4 m below the surface, had a beamwidth (at the down 6 db level) of 6° horizontally and 15° vertically. The tag could be detected as far as 2.3 km with this equipment.

During tracking operations, contact with the transmitter was maintained by continually scanning in bearing and tilt about 6° on either side of the last observed position. The ship was maneuvered to maintain a distance of approximately 1 km from the tagged fish to avoid affecting the behavior of the fish. Because of errors in estimating the distance of the transmitter by its signal strength and confusion in operations, however, there were a few occasions when the distance between ship and transmitter was as little as 200 m or was greater than 2.3 km, the detectable range.

During tracking the following data were recorded: time of day, transducer bearing, transducer tilt, ship's course, ship's speed, receiver gain setting, and signal strength. Also recorded were all orders directing the ship's movements and the time the orders were given. Ship's course was read to the closest degree from a repeater of the ship's gyrocompass. Speed was measured to tenths of knots by an electromagnetic underwater log. Signal strength was measured by an oscilloscope. These data were scheduled to be taken at 5-min intervals. In practice the recording interval ranged from 2 to 10 min, but most were at 5 min. The officer on watch was asked to plot the ship's position every hour but he often did so more frequently. Positions were determined by radar at Kaula Bank and by loran A at Penguin Bank (Fig. 2).

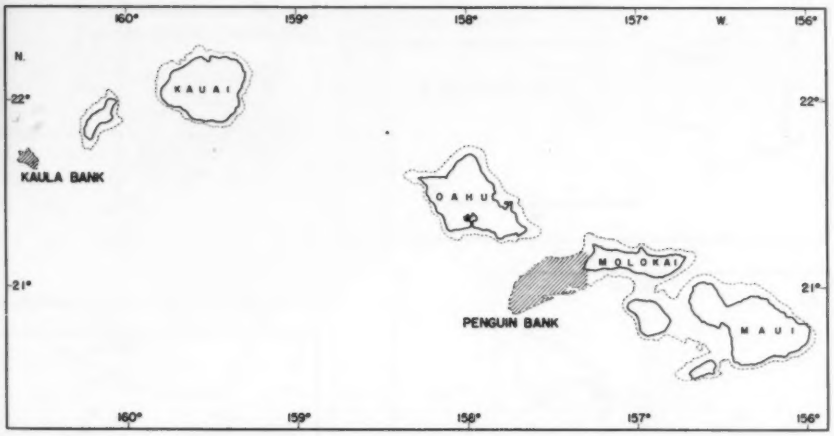

FIG. 2. Location of Kaula Bank and Penguin Bank.

Because the fish were likely to be within 1 km of the ship and certainly not farther than 2.3 km and because the navigational error term did not justify further refinement, the positions of the fish were considered to be the same as the ship's when the data were analyzed.

RESULTS AND DISCUSSION

The first skipjack tuna was tagged and released at 2:52 PM on August 30. It was estimated to be 44 cm long. It was in a school that had been sighted

at 1:05 PM at Kaula Bank and had been pursued for 28 km (Fig. 3) before the fish was tagged. The marked fish was under continuous surveillance until 8:00 AM, August 31, when contact was lost. It was found at 9:32 AM, August 31 and tracked continuously until 10:31 AM, September 1, when the sonar broke down. A trip was made to Honolulu to repair the sonar, and the ship was back at Kaula Bank at 6:30 AM, September 3, at which time search for the signal

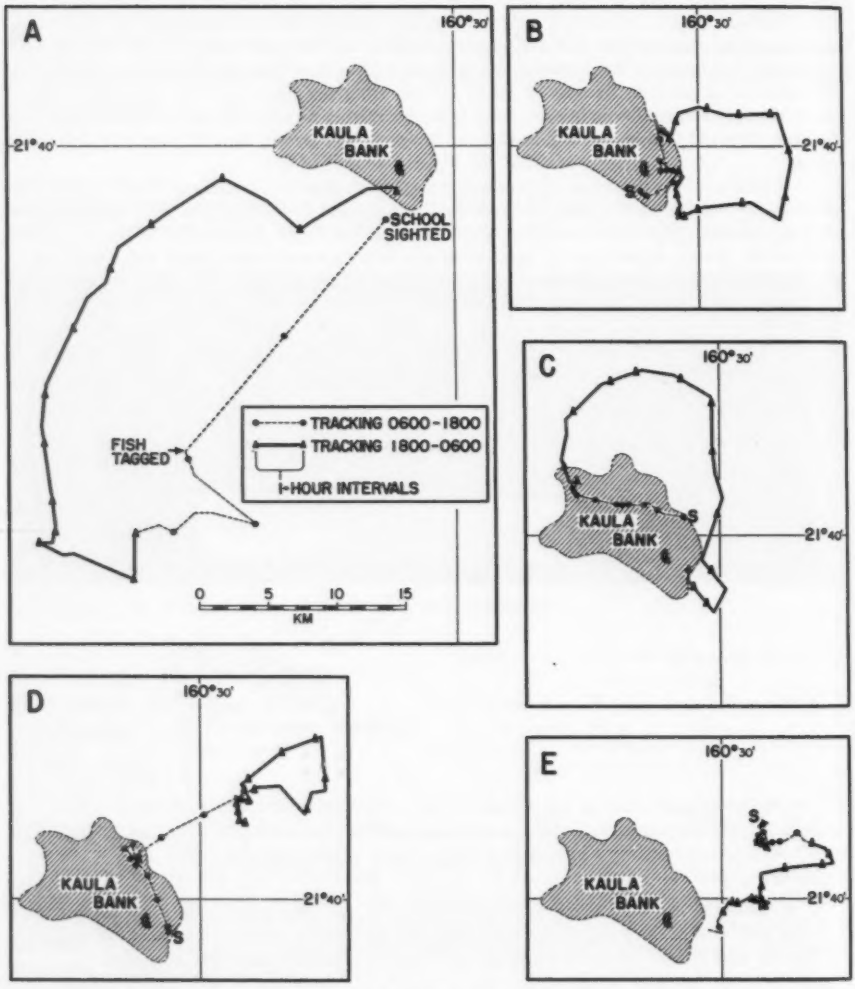

FIG. 3. Track of tagged skipjack tuna at Kaula Bank: (A) from 2:52 PM, August 30, to 6:00 AM, August 31; (B) from 6:00 AM, August 31, to 6:00 AM, September 1; (C) from 7:38 AM, September 3, to 6:00 AM, September 4; (D) from 6:00 AM, September 4, to 6:00 AM, September 5; (E) from 6:00 AM, September 5, to 7:30 AM, September 6.

from the skipjack tuna was started. At 7:38 AM contact with the transmitter was resumed. On September 4 the fish was lost from 3:45 AM to 5:42 AM. From 6:38 AM to 9:12 AM that same morning acoustic contact was broken because the fish was too close to shore for the ship to follow. It was tracked nonetheless because the school it was in was visible. Contact with the fish was lost twice more: 4:30–4:55 AM on September 5 and 1:57–3:02 AM on September 6. Tracking was voluntarily ended at 7:30 AM, September 6.

We terminated tracking the skipjack tuna at Kaula Bank to collect comparative data from another bank. At 12:55 PM on September 8 a second skipjack tuna about 40 cm long was tagged at Penguin Bank. This fish was tracked until 12:45 AM, September 9, at which time it was lost.

The fish at Kaula Bank exhibited a pattern. At about nightfall each day, the fish left the bank to follow an irregular and wide-ranging path offshore during the hours of darkness; by about sunrise it was back at the bank and it remained there until night (Fig. 3). Even though the route differed from night to night the fish was back at the bank five of the six mornings it was observed. On the excepted morning it was 9 km from the bank and it remained there throughout the day. It was at the bank the following morning after a night of traveling. The fish at Penguin Bank also left the bank at the end of the day (Fig. 4).

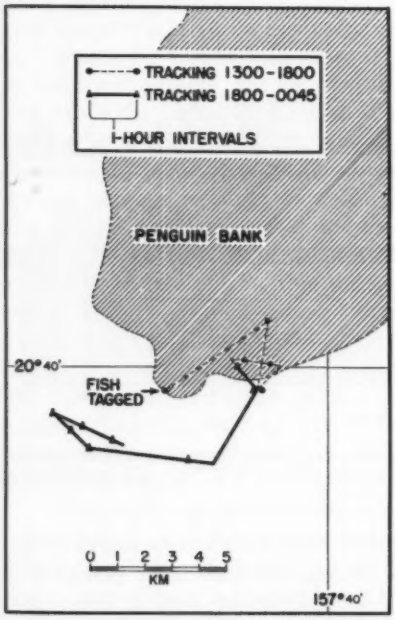

FIG. 4. Track of tagged skipjack tuna at Penguin Bank.

On its nocturnal journey the fish at Kaula Bank covered distances of 106, 31, 40, 36, and 25 km, in that order. The first night's journey was considerably longer than the others. The long pursuit before tagging is suspected as a cause of the atypical length of that run.

During the day a school of skipjack tuna was often visible at the position whence the transmitter signals were being received. On several occasions, during both day and night, the ship inadvertently approached within 200 m of the tagged fish. When the sonar was switched to the active mode at these times, many targets were observed in the vicinity of the tag. I assumed from these observations that the tagged skipjack tuna rejoined their schools and that their behavior was typical and representative of other skipjack tuna.

To investigate in more detail the daily periodicity evident from the fish's track, the means and variances of the distance traveled for each hour of the day were calculated. The variances (Fig. 5A) fell into three levels: less than 1.1,

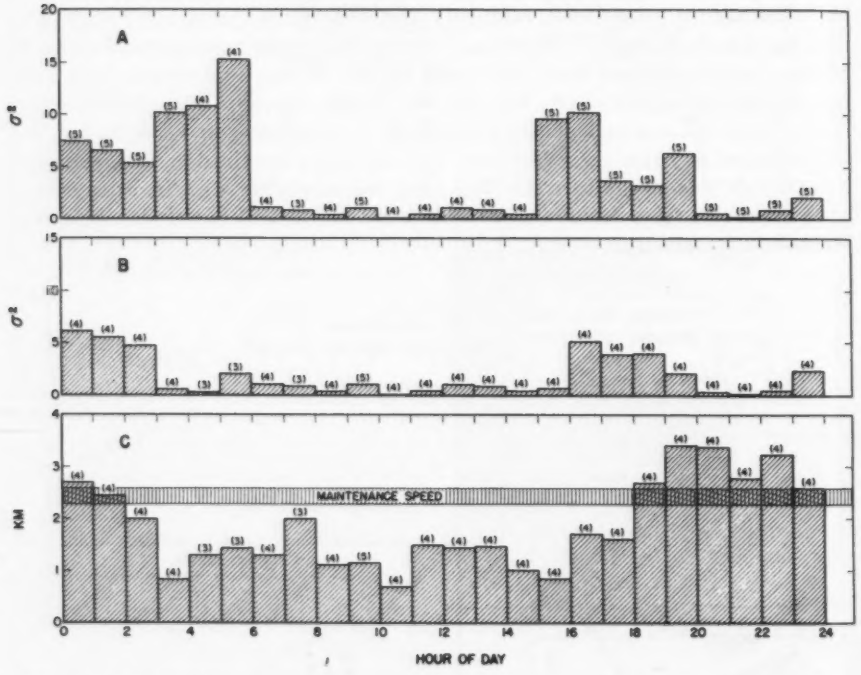

FIG. 5. Daily swimming pattern: (A) variance of distance traveled per hour calculated from all data; (B) variance of distance traveled per hour excluding data of first 15 hr; (C) mean distance traveled per hour.

2.0–7.5, and greater than 9.5. At the highest level the primary source of variation was the unusual distance traveled during the first 15-hr period after tagging. The means and variances were then recalculated without these extra high values (Fig. 5B and C).

The prominent feature of the histogram of distance traveled per hour (Fig. 5C) is the higher level from 6:00 PM to 2:00 AM. The increase, occurring as it did at 6:00 PM, immediately suggested that it was triggered by sunset,

which occurred between 6:09 and 6:17 PM on the days concerned. The data from individual days, however, show that the start of the increase occurred between 4:00 and 7:00 PM. This 3-hr spread is reflected in the higher level of variances during that period (Fig. 5B). Another period of higher variances from 12:00 AM to 3:00 AM is obvious in Fig. 5B. This is the period during which the changeover from the high rate of travel to the lower rate began.

The distances traveled were based on positions of the ship so that they indicate the net hourly movements of the ship and not necessarily those of the tagged fish. Under the conditions of collection of the data, the distance traveled by the ship will always be less than that traveled by the fish but will approach that of the fish as the route of the fish approaches a straight line. The distance traveled by the ship could be zero if the fish made many turns. Depending on the circumstances, then, changes in the distance traveled (Fig. 5C) could mean changes in swimming speed, changes in swimming pattern, or both.

For hydrodynamic and physiological reasons tunas swim continuously. The swimming occurs at various minimum speeds. The minimum speed for skipjack tuna is not known but it has been calculated for *Euthynnus affinis*, a closely related species (Magnuson, 1970). For an *E. affinis* of 44 cm fork length minimum speed is 2.3–2.6 km/hr. If we assume that the tagged tuna did maintain this speed, then from 2:00 AM to 6:00 PM, when the rate of travel was below this level, its swimming pattern must have included much turning.

When the hydrophone is at 0° (aimed horizontally) to 7½° vertical tilt to receive the signal, the fish may be at any depth from the surface to about 500 m. If the signal is received at a tilt greater than 7½°, however, the fish cannot be within 4 m of the surface. The percentage of tilt angle records that exceeded 7½° was calculated for every hour of the day (Fig. 6). Since the intervals between readings were fairly constant, this percentage also represents the percentage of time that the fish was away from the surface for certain. From 5:00 AM to 8:00 PM the fish at Kaula Bank (Fig. 6A) spent an average of 29% of the time away from the surface. For the remaining hours the average was 2.6%. This average should be less because the readings greater than 7½° between midnight and 2:00 AM were obtained one night when the ship inadvertently passed directly over the fish. From the time it was tagged until 5:00 PM the fish at Penguin Bank spent a large proportion of its time away from the surface (Fig. 6B). Beyond 5:00 PM until it was lost, however, the hydrophone was aimed horizontally 92% of the time and was tilted a maximum of 5° the rest of the time.

The following picture of the behavior of skipjack tuna that are associated with banks can be drawn from these results. They have a general daily pattern. They usually spend the day at the bank, where they swim to and fro and are away from the surface a good part of the time. This type of swimming is probably associated with searching for food and feeding. Later in the day, within a couple of hours of sunset, they leave the bank and swim with few changes in direction until approximately 2:00 AM, when they seem to adopt a more erratic swimming pattern. Although they leave the bank by different routes

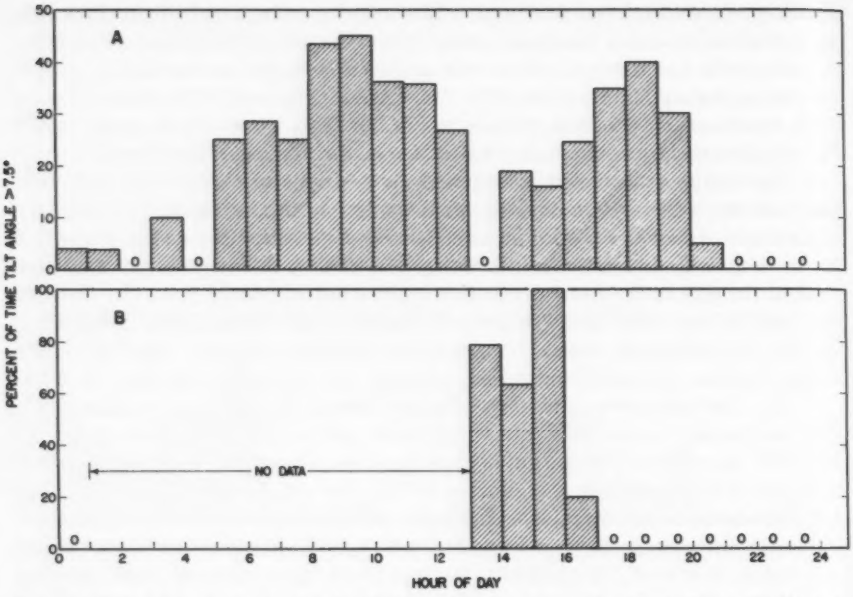

FIG. 6. Daily pattern of swimming depth: (A) skipjack tuna tagged at Kaula Bank; (B) skipjack tuna tagged at Penguin Bank.

from day to day, they are usually back at the bank by sunrise. They are presumed to be close to the surface throughout the night.

The repeated returns to the same spot by the fish at Kaula Bank after journeys of 25–106 km by various routes imply that skipjack tuna can navigate. Their consistent arrival times suggest that they have a sense of time. It is interesting to note that from 3:00 AM to 6:00 AM of the first morning after it was tagged, when it was unusually far from the bank, the tuna at Kaula Bank averaged 8 km/hr, seven times its average speed for that time of the day, as if it were compelled to arrive at the bank by a certain time.

On November 30, 1969, a skipjack tuna (52.2 cm fork length) marked with a Floy dart tag was caught at Kaula Bank (R. N. Uchida, personal communication). It was tagged on May 19, 1969, at 22°01′N and 155°55′W, a position 519 km from Kaula Bank. This recovery and the fact that skipjack tuna over 60 cm long seldom occur at banks indicate that they are not lifetime residents of a bank but come for an undetermined duration and leave.

Skipjack tuna over 60 cm long have different food preferences from those less than 50 cm long (Yuen, 1959). The swimming behavior of the fish tracked at Kaula Bank and Penguin Bank was typical of foraging tuna. This evidence suggests that food preferred by the small but not by the large tuna is found at the banks and that the small tuna frequent the banks for the purpose of feeding.

ACKNOWLEDGMENTS

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Relation of the Phytoplankton to Turbulence and Nutrient Renewal in Casco Bay, Maine[1]

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HULBURT, E. M., AND N. CORWIN. 1970. Relation of the phytoplankton to turbulence and nutrient renewal in Casco Bay, Maine. J. Fish. Res. Bd. Canada 27: 2081–2090.

A diatomaceous phytoplankton remains at fairly high densities, 10^2–5×10^3 cells/cm^4, from the end of winter to the end of summer in Casco Bay. During spring the algal populations deplete the nutrient content of the water, particularly nitrate, except in the deep water at the entrances to the bay. Here appreciable quantities of nitrate persist into midsummer. These may be carried inward by influxes of deep water and then may be mixed upward to support the continued growth of diatoms, although the change from the spring diatoms belonging to the genera *Thalassiosira* and *Chaetoceros* to the summer dominant *Skeletonema costatum* is considered to depend on temperature and light changes. The high concentrations of *Skeletonema*, often exceeding 10^3 cells/cm^3, are shown to be enough for the maintenance and growth of several filter-feeding animals.

Received June 16, 1970

INTRODUCTION

HORIZONTAL MOVEMENT of cold, deep water into Casco Bay, one of the embayments of the coast of Maine, has been deduced by comparing the large amount of heat entering the water surface with the small amount observable in the temperature increase of the water mass of the bay (Hulburt, 1970). Vertical mixing of surface and deep water, respectively cooling and warming these portions of the water column, has been found to be more marked where currents are strong, in narrow embayments and between closely spaced islands, and less marked in open water along the Maine coast (Hulburt, 1968b). These hydrographic features will be shown, in the following pages, to affect the distributions of phytoplankton and nutrients in Casco Bay and to account for the prolonged abundance of diatoms from late winter to the end of summer.

METHODS

Phytoplankton samples were preserved with formaldehyde (3–100 cm^3 of sample) and were concentrated in the following manner. First, 90 cm^3 were centrifuged in six 15-cm^3 tubes. After the supernatant was removed, the residues in the six tubes were transferred to two tubes, and enough sample was added to bring the total volume of a processed sample to 103 cm^3. The concentrate from these tubes, after final centrifugation, was spread upon a slide under a rectangular cover slip. A portion of the area of the cover slip equivalent to 10 cm^3 of original sample was scanned

[1]Contribution No. 2511 from the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543.

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and the species enumerated. When a species was very abundant, less than the equivalent of 10 cm^3 was scanned, although this amount was examined for the rarer forms. More than 10 cm^3 of the original sample were examined when cell densities were low in the fall.

Phosphate and nitrate samples were preserved with mercuric chloride and analyzed by the methods described by Murphy and Riley (1962) and Wood et al. (1967).

All samples were taken with a Van Dorn sampler, from the depths shown in Fig. 2–4.

Water transparencies were measured by Secchi disc. These measurements were converted to extinction coefficients by dividing 1.7 by the depth (in meters) at which the disc could just be seen (Curl, 1959). The extinction coefficients were converted to values for the compensation depth (the depth at which photosynthesis and respiration are equal). Ryther (1956, fig. 5) presents the relation between daily relative photosynthesis and the product of depth and extinction coefficient for an incident radiation of 802 cal/cm^2 per day. An alternate scale is given in this figure showing the relation between photosynthesis and percentage of incident radiation, so that curves for photosynthesis at 400 and 200 cal/cm^2 per day of incident radiation could be drawn. Where these curves reach a value of $\frac{1}{16}$ of maximum photosynthesis, respiration and photosynthesis are considered equal, and the depth for a given extinction coefficient may be found. Compensation depths at radiation values between 400 and 800 cal/cm^2 per day were obtained by interpolation.

Samples of surface water were enriched with Guillard's medium *f* (Guillard and Ryther, 1962) in dilutions as shown in Table 3. The samples were suspended at 0, 10, 20, 30-ft depths from a rowboat anchored between Great Chebeague Island and Long Island in Casco Bay. After a period of 3 or 4 days the phytoplankton in the samples was counted to determine its increase over the initial count. The same technique, using cultures of several species, has been used by Talling (1955) at Lake Windermere.

In Fig. 1 a map is given of that portion of Casco Bay where observations were made.

RESULTS

Table 1 presents surface densities of total cells and of dominant species for all seasons except midwinter at Casco Bay. The dominant species, all diatoms, made up 85% or over of the populations, except on March 2. The range of abundance during spring and summer, seasons with adequate coverage, was such that $\frac{1}{3}$ of the samples exceeded 1000 cells/cm^3 and $\frac{2}{3}$ exceeded 100 cells/cm^3 (one sample was lower than 100 cells cm^3).

Table 1 shows that there was a succession of dominant forms from *Thalassiosira nordenskiöldii*, found at the end of one winter, to *Chaetoceros socialis* and *C. debilis*, observed during springs of two years, and thence to *Skeletonema costatum*, present during three summers. On March 2, 1968, all four dominants were present but only *T. nordenskiöldii* was abundant. *Skeletonema* was present on that date as it was on other dates in spring and fall, but only in summer did it become abundant. Its abundance lasted to the end of summer, for on August 30, 1966, its numbers ranged from 705 to 1965 cells/cm^3 at stations B–F.

The profiles of Fig. 2 illustrate the succession from *Thalassiosira* to *Chaetoceros* to *Skeletonema* during spring and summer. They show that these species occurred throughout the bay when they were dominant. *Leptocylindrus minimus* was abundant on only one survey and then only in the inner bay.

In spring the phytoplankton extended to the bottom in the entrances to the bay in densities that differed rather little from those of the surface populations (Table 2). In summer several instances of reduced numbers at the bottom occurred, for on three occasions, cell numbers were less than 10/cm^3.

FIG. 1. A portion of the coastline of the Gulf of Maine (*upper*). Central and western Casco Bay (*lower map*). Station locations are marked B–K, A not being shown but lying 4 miles southeast of Halfway Rock. The 30-m depth contour is indicated by the broken line.

Nevertheless, on the seven other occasions the range in total numbers was 39–283/cm³ at 25–30 m, less, though not greatly less, than the range at the surface, 80–1080/cm³.

Although the phytoplankton extended to 30 m at the bay entrances, its in situ growth was restricted to depths much closer to the surface. In Table 3 is shown the increase of *Skeletonema* in enriched samples suspended at various depths. Growth was at a maximum at the surface and decreased to none at all at 9 m.[2]

The depth at which photosynthesis should equal respiration (compensation depth) and no growth should occur may be obtained from Ryther (1956) (see methods) using the values for radiation and extinction coefficient in our

[2]At Lake Windermere, Talling (1955) obtained similar results. No growth occurred below 7 m. The turbidity of the water was about the same as in the inner parts of Casco Bay (Talling, 1957).

Table 1. Surface densities of total cells and dominant species, in cells/cm³, at Whaleboat Island except where otherwise indicated. All dates on which a sample was taken anywhere in Casco Bay are included.

Date	Total count	*Skeletonema costatum*	*Leptocylindrus minimus*	*Chaetoceros debilis*	*Chaetoceros socialis*	*Thalassiosira nordenskiöldii*	*Rhizosolenia delicatula* or *Guinardia flaccida* (+)
				1966			
July 16[a]	2861	2840		3			
25	523	480		1		.2	
Aug. 30	716	705					
Oct. 16[a]	16	3					5+
Nov. 13[b]	18	1					14+
				1967			
Apr. 15[b]	459			84	366		
27[b]	13	.4		6	1		
May 7	321			165	144	2	
June 10	336		213	116			
July 11	1090	1080		8			
13[a]	5779	5600		60			
16[a]	4688	4600	4	10			
19[a]	747	720	6	2			
20[a]	581	564	6				
23[a]	860	832	20				
Aug. 6[c]	842	830		6			
Nov. 10	148						114
				1968			
Mar. 2	104	.7		1	2	65	
Apr. 18	1586			58	1420	63	
June 6	162	1	.8	152			
17	649	28	1	564			
23	279	46		198			
July 3	2867	2800		27			
16	1082	983					

[a]At Great Chebeague Island.
[b]At Cousins Island.
[c]At Pumkin Nob.

Table 3. The compensation depth at the time of the experiments was 6–7 m, supporting, roughly, our experimental results.

The restriction of in situ growth to the near surface water was due to the turbidity of the bay. Average values during summer for the extinction coefficient and the corresponding compensation depth are shown in Table 4. Within the confines of the islands (stations C–F), the compensation depth was less than 10 m.

The abundance of the plankton, though restricted in its actual growing to the near-surface water and thence distributed downward, must have depended in early spring on the quantity of nutrient present and, later, on renewal

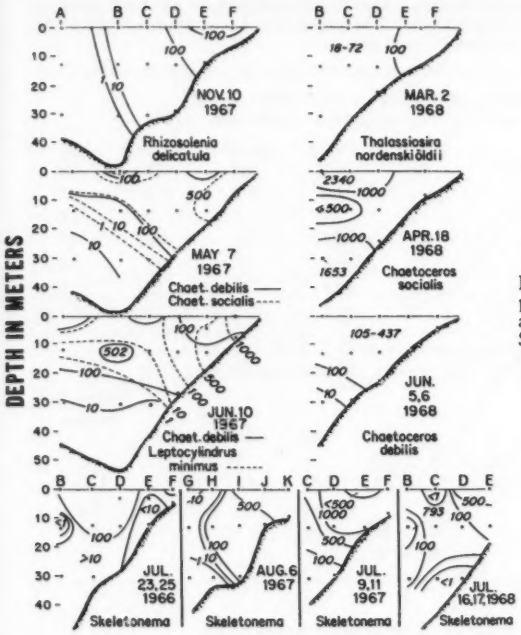

FIG. 2. Distribution of dominant phytoplankton species in cells/cm³ along sections A–F and G–K. Sampling depths indicated by solid circles.

TABLE 2. Densities of dominant species in number of cells per cm³.

Stations:	C		D	
Depths:	0 m	30 m	0 m	30 m
Nov. 10, 1967	74	21	114	56
Mar. 2, 1968	64	–	65	46[a]
Apr. 18, 1968	1860	1213	1420	648[a]
May 7, 1967	129	53	165	103[a]
June 6, 1968	182	58	152	283[a]
June 10, 1968	80	3	116	98
July 9–11, 1967	563	39	1080	63
July 17, 1968	.5	.3	983	< .1
July 23–25, 1966	246	82	483	–
Aug. 6, 1967	830[b]	162[b]		

[a]At 25 m.
[b]At station I.

of nutrient from deep water. Phosphate and nitrate concentrations were fairly high in the ocean in November and at the end of winter but were reduced toward Merepoint, station F (Fig. 3–4). Both nutrients decreased in surface waters during spring, nitrate more so than phosphate. A peculiarity in the phosphate

TABLE 3. Growth of the dominant species, *Skeletonema costatum*, in enriched samples suspended at indicated depths between Great Chebeague and Long islands. Values are number of cells per cm³.

(A) Four 3-day experiments in July 1967; initial enrichment, 30 μg-at P/L; extinction coefficient, 0.42

Dates		16–19	19–22	20–23	23–26	Avg
Solar radiation, cal/cm² per day		338	380	388	478	398
Initial count		5	0.7	0.5	0.8	2
Final count	Control (0 m)	7	12	2	2	6
	0	90	75	42	93	75
	3		21	19	45	28
	6	8	4		11	8
	9	5	1	3	3	3

(B) One 4-day experiment, July 3–7, 1968; average solar radiation, 382 cal/cm² per day; extinction coefficient, 0.48

								Maximum counts
Enrichment, μg-at P/L		0	1	10	20	30	40	
Initial count		3	3	3	3	3	3	
Final count	0 m	4	27	38	125	92	105	125
	3		14		82			82
	6		12	40	26			40
	9		6	6				6

TABLE 4. Average extinction coefficients in Casco Bay and corresponding values of the compensation depth. Extinction coefficients are averages of measurements from the surveys of June and July 1966–68. Compensation depths are calculated for an incident radiation of 500 cal/cm² per day, an approximate average for summer conditions.

Station	Extinction coefficient	Compensation depth (*m*)
A	0.21	15.3
B	0.25	12.8
C	0.33	9.8
D	0.38	8.5
E	0.46	7.0
F	0.68	4.7

distribution was the slightly increased amounts near the bottom in the inner bay (stations E, F, and K) in summer, contrasting with the absence of detectable nitrate in the same places. But in the deep water small amounts of nitrate persisted from late spring to midsummer.

DISCUSSION

The disparity in the distributions of the nutrients probably was the result of the short length of time, several weeks, necessary for production of phosphate

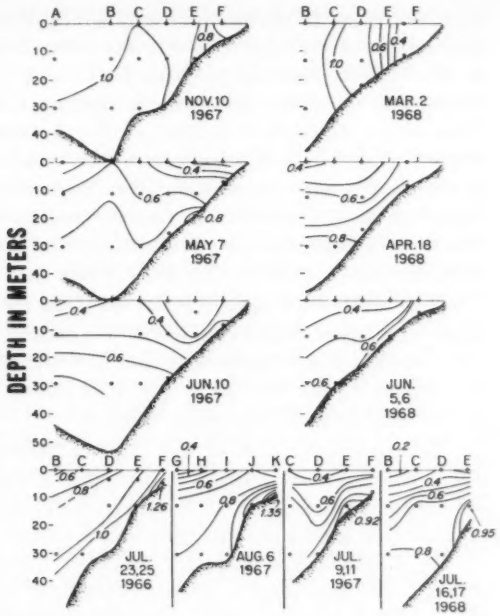

FIG. 3. Distribution of inorganic phosphate in μg-at P/L along sections A–F and G–K (see Fig. 1). Sampling depths indicated by solid circles.

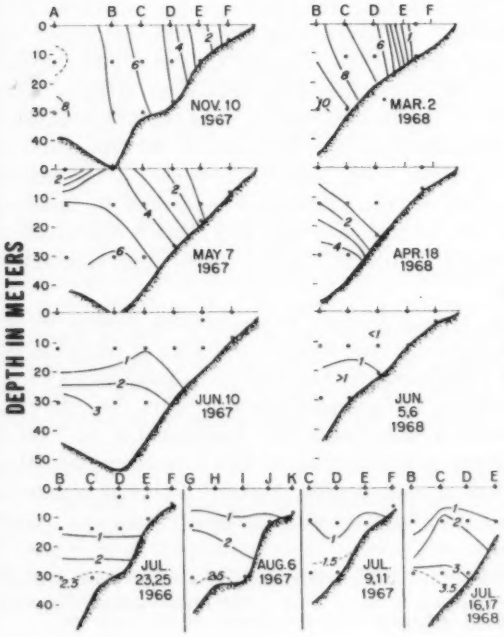

FIG. 4. Distribution of inorganic nitrate μg-at N/L along sections A–F and G–K (see Fig. 1). Sampling depths indicated by solid circles.

from decaying plankton (Cooper, 1935; Vaccaro, 1965) and the long time required for nitrate, several months (Von Brand et al., 1937; Vaccaro, 1965). In the bottom water at stations E, F, and K, less affected than the seaward stations by the deep water outside the bay, excess phosphate could accumulate from decay of organic matter in the bottom sediment during summer. In contrast, the source of nitrate at this time was outside the bay, and its presence in the deep water about the entrances and absence at stations E, F, and K indicated the accessibility of the seaward stations to intrusion of deep water. Heat budget calculations (Hulburt, 1970) show such intrusion, which would replenish the nitrate in the deep water of the bay.

The downward extension of diatoms far below the compensation depth must have been due, in addition to sinking, to the active mixing about the outer islands and entrances to the bay (Hulburt, 1968b). This mixing would also transport the nitrate in the deep water upward, supporting the growth of the diatoms at the same time.

In the central part of the Gulf of Maine south and east of Casco Bay, where there are no sources of turbulence other than wind and temperature differences greater than 10 C (Rakestraw, 1933) occur in the upper 30 m in summer, the spring growth, dominated by the diatoms *Thalassiosira* and *Chaetoceros*, is followed by a sparser plankton, dominated usually by the coccolithophore, *Coccolithus huxleyi* (Gran, 1933; Gran and Braarud, 1935; Bigelow et al., 1940). The continued abundance of the diatom *Skeletonema costatum* through summer at Casco Bay, where temperature differences at the entrances were 5–8 C/30 m, shows, by comparison, the distinctive effect that enhanced turbulence and concomitant nutrient renewal may have on the flora. This comparison is of more than local interest, since abundant coastal or shallow water populations (10–1000 cells/cm^3) in the western North Atlantic are dominated by diatoms and the meager populations of the open sea (1–10 cells/cm^3) by coccolithophores (Hulburt, 1962, 1964, 1966, 1968a). However, in the coastal regions embraced by these studies marked turbulence may only be assumed to be the cause of large populations, whereas in Casco Bay there is real evidence for the existence of active mixing (Hulburt, 1968b, 1970) and consequent renewal of nutrient supplies.

Although the change from spring diatoms to *Coccolithus huxleyi* in the central Gulf of Maine may be due to a reduction of nutrient in summer, the change from *Thalassiosira* and *Chaetoceros* to *Skeletonema* in summer at Casco Bay would appear to be dependent on temperature and light. On March 2 the three diatoms were all present in water of 0–2 C. Experiments by Jitts et al. (1964) show that *Thalassiosira* grows at 2 C or less in culture, but that *Skeletonema* needs a temperature several degrees higher.[3] The abundance of *Skeletonema* in summer and rarity of *Thalassiosira* (its absence from our counts

[3]The winter bloom of *Skeletonema* in Narragansett Bay starts at a temperature of about 5 C and continues as the temperature decreases to about 2.5 C (Pratt, 1965), temperatures appreciably higher than at Casco Bay on March 2.

does not mean that it was absent from the bay water) may have been fostered by the somewhat greater growth rate of *Skeletonema* than *Thalassiosira* at high light intensities (300–600 cal/cm² per day) between 10 and 20 C, as found by Jitts et al. (1964). Unfortunately no information on *Chaetoceros debilis* is available.

The continued abundance of diatoms from spring to the end of summer is of great importance to the sedentary, filter-feeding animals, for extensive mussel and soft clam flats occur between Great and Little Chebeague islands and between Cousins Island and the mainland (Fig. 1). The quantity of cells necessary to satisfy the respiratory needs of a filter-feeding animal depends on the ratio of the amount of water pumped or filtered to the amount of oxygen consumed. When natural sea water, which contains no added cells or particles, is passed through the animal, is measured, and its O_2 depletion determined, a ratio of 3.35 liters pumped per ml O_2 consumed is obtained for the American oyster (Galtsoff, 1964, tables 23–25; Loosanoff, 1958, table 1), though lower ratios were found by Collier (1959) for the oyster. But when cells or particles are added to a fixed volume of water and the reduction in their numbers is used to compute the quantity of water passed through the animal and this quantity is in turn compared with the respiration in a similar volume of water without added cells or particles, a ratio of about 10 is obtained for the scallop *Pecten irradians* (Chipman and Hopkins, 1954; Van Dam, 1954). Using a similar method Jorgensen (1960) obtained an average ratio of 10.8 for the mussel *Mytilus edulis* and a higher ratio for the oyster. Choosing an intermediate ratio, 7.0, one finds that 2000 cells/cm³ of *Skeletonema*, the average of July–August values in Table 1, are more than enough to satisfy the respiratory requirements of *Mytilus*. (7.0 (liters pumped/ml O_2) × 2,000,000 (cells/liter of *Skeletonema*) × 516 μ^3 (volume of *Skeletonema*) × 1 (density of protoplasm) × 23% (% dry organic matter, Ketchum and Redfield, 1949) = 1.66 mg of organic matter per ml O_2, which is twice that necessary for the consumption of 1 ml O_2 (Jorgensen, 1952).) In spite of the uncertainty in the choice of ratio the calculation does suggest an adequate amount of food in summer for maintenance and growth of mussels. Certainly it is difficult to see how these animals would exist if exposed to cell concentrations $\frac{1}{100}$ those of Casco Bay — the concentrations that occur in the offshore Gulf of Maine in summer. The adequacy of cell concentrations above 1000/cm³ is further indicated by a study by Matthiessen (1960) of a salt pond on Cape Cod. Here, an increasing growth rate of the soft clam, *Mya arenaria*, was correlated with increasing amounts of phytoplankton ranging from 1000 to 7000 cells/cm³.

ACKNOWLEDGMENTS

The surveys of May 7, June 10, and November 10, 1967, were made aboard the boat belonging to Mr F. Symonds; the survey of March 2 and April 18, 1968, were done from craft belonging to Mr A. Burgess and Mr J. E. Smith. The authors are indebted to Messrs Symonds, Burgess, and Smith for their assistance. The remaining surveys were done from the senior author's own boat.

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OBITUARY

John Stanley Milton Harrison

1921–1970

John Stanley Milton Harrison

1921–1970

It is with deep regret that the death of Mr J. S. M. Harrison, a valued long-time engineer with the Fisheries Research Board and the Fisheries Service of the Department of Fisheries and Forestry, is reported. Mr Harrison died in an automobile accident on April 19, 1970, in Vancouver, B.C. He was 49 years of age. He is survived by his wife and three children.

Mr Harrison was born and educated in Vancouver, and graduated from the University of British Columbia in 1943 with a Bachelor's degree in mechanical engineering. He joined the Royal Canadian Electrical and Mechanical Engineers and served as a Lieutenant in England and in northwestern Europe.

On demobilization in early 1946, he joined the Fisheries Research Board of Canada as a research engineer. Early efforts included work as a member of a team in the study and assessment of the ice–salt bunker car system that was in use on Canadian railroads for the preservation of temperature-dependent food products. Work followed in the design development and testing of mechanically refrigerated railroad cars. The success of these efforts is attested to by the present widespread use of such equipment in the rail transport of fresh and frozen food products.

It was in fishing-vessel refrigeration, however, that Mr Harrison made the greatest contribution and enjoyed a most enviable national and international reputation in the fishing industry, shipbuilding, and government circles, as an expert in this field. He travelled widely, both in Canada and abroad, presenting papers, advising and consulting in almost all aspects of vessel refrigeration, especially refrigerated seawater systems, which, incidentally, are coming into use in many parts of the fisheries world, both on catching and carrier vessels.

Mr Harrison left the Fisheries Research Board in 1962 and joined the Industrial Development Service of the Department of Fisheries, subsequently joining the Inspection Service in 1967 to guide its engineering activities on Canada's Pacific coast.

During Mr Harrison's long service with the Fisheries Research Board and the Fisheries Service of the Department he was co-designer and co-holder of patents on equipment for vessel refrigeration, vessel unloading systems, canning machinery, and processing devices. In recent years he was engaged in the development of brine-spray systems for chilling and freezing fish at sea, the engineering design aspects and operation of facilities for oyster depuration, and the development of a system for refrigerating vessels in which a unique extruded aluminum evaporator coil is integrated with the aluminum hold lining so providing an extended cooling surface effect.

Mr Harrison's expertise in fisheries engineering and his cooperative activities will be greatly missed by the fishing and allied industries, as well as by all who worked with him over the past years.

S. W. Roach
M. A. Foley

NOTES

Hyperplasia in an Estuarine Bryozoan Attributable to Coal Tar Derivatives

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AND

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POWELL, N. A., C. S. SAYCE, AND D. F. TUFTS. 1970. Hyperplasia in an estuarine bryozoan attributable to coal tar derivatives. J. Fish. Res. Bd. Canada 27: 2095–2096.

Hyperplasia of ovicells was induced experimentally in *Schizoporella unicornis* (Johnston) by placing colonies with normal numbers of ovicells in close proximity to coal-tar derivatives in an estuary. The hyperplasia is attributable to one or more carcinogens in the petrochemicals.

Received October 15, 1970

THIS IS a preliminary report on experimental induction of hyperplasia in the estuarine bryozoan *Schizoporella unicornis*. This is a common species in Willapa Bay, Washington, where it occurs in close association with commercial beds of the Pacific oyster, *Crassostrea gigas* (Thunberg), where salinities remain above 15‰.

Comparison of colonies from areas in the bay with no evidence of petroleum derivatives and others from four areas in which derivatives were evident indicated the presence of abnormal colonies in the latter areas. In normal colonies (Fig. 1) the ovicells, or brood chambers, are regularly distributed throughout the breeding zone, one to each individual and distal in position. On completion, these ovicells always contain developing embryos. Normal colonies occur throughout most of the bay. In abnormal colonies, on the other hand, the ovicells are irregularly distributed and number 2–4 to each individual (Powell, 1970), usually comprising a distal (normal) chamber as well as two or three additional chambers, variously positioned around the orifice (Fig. 2). The additional chambers do not usually contain embryos.

One of the areas where petroleum derivatives are evident is the Nahcotta boat basin. The slick in this basin is derived from several sources, especially creosote leaching continuously from pressure-treated dock pilings, gas and diesel oil spilled from small craft, and engine oil pumped from boat bilges.

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At the other three areas, Stony Point, Stackpole, and Parcel "A," Nahcotta, stations are maintained by the Washington State Fisheries Department concerning oyster development. The young oysters are stocked in trays with wooden frames pressure-treated with creosote and wire-mesh bottoms coated with a mixture of asphalt and creosote.

In the boat basin, abnormal colonies encrust styrofoam floats and the bases of boat fenders, at a depth of 3–12 inches. At the oyster stations, they occur only on the oyster shells, in close proximity to the coal-tar derivatives.

In experiments in the bay from July to October 1970, we transferred normal colonies on shells taken from oyster reefs to the boat basin and two of the oyster stations to find whether abnormal colonies would develop. In the boat basin the encrusted shells were threaded on nylon rope and suspended just below the water surface. At the oyster stations the shells were either placed on the trays or fastened to them with polyvinyl-chloride (PVC) string.

Abnormal colonies (e.g., Fig. 3) were induced in the boat basin in 9 days, and at one of the oyster stations in 7 days.

These experiments have three important implications:

1. Our observations document for the first time a reproductive response by a marine invertebrate to the presence of coal-tar derivatives in the environment. The rapid development of the hyperplasia suggests that *S. unicornis* may be useful in monitoring qualitatively, and perhaps even quantitatively, oil pollution in coastal estuaries.

2. The uncontrolled growth of the ovicells was presumably triggered by the presence of one or more of the several carcinogens widely known to be present in coal tar, e.g., 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, and methylcholanthrene. Both dibenzanthracene and methylcholanthrene have a hormonal effect in vertebrates, dibenzanthracene exhibiting oestrogenic properties (Bäumler, 1968, p. 90). Possibly, dibenzanthracene has blocked off the ovarian hormone (Silén, 1944), which in this and other ovicellate bryozoans ordinarily regulates development of the ovicell.

3. Though similar abnormalities have not been reported among effects of massive oil spillages on coastal faunas, e.g., from the *Torrey Canyon* and at Santa Barbara, California, and Chedabucto Bay, Nova Scotia, the studies have been largely observational and based on population mortalities. Is it possible that these spillages have induced similar, but undetected, abnormalities in this and other groups of invertebrates?

Acknowledgments — We thank Dr V. Tartar, Nahcotta, Washington, for helpful suggestions.

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(*Figures opposite*)

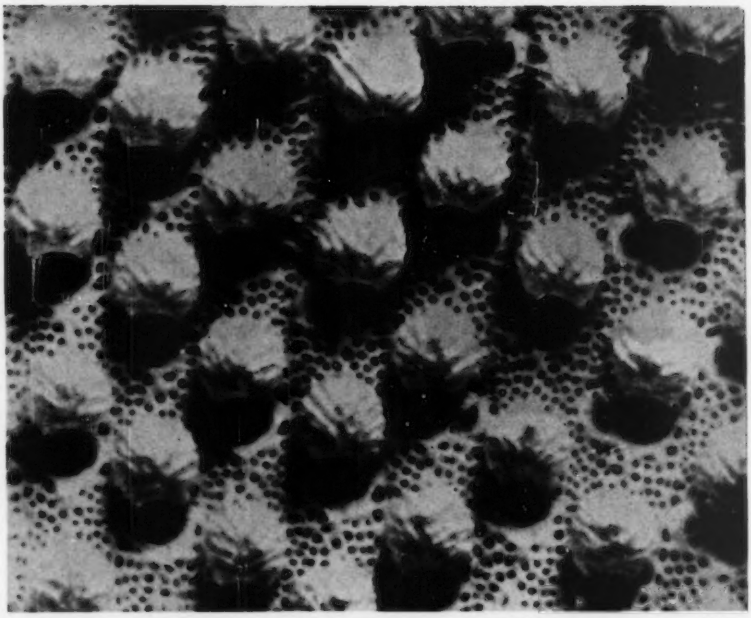

FIG. 1. A normal colony of *S. unicornis* showing the regular arrangement of ovicells (brood chambers), one to each individual. From Long Island Reserve, Naselle River, Willapa Bay. ×44.

FIG. 2. Highly advanced hyperplasia in colony found on oyster tray at Stony Point, Willapa Bay, September 1, 1970. Tray, treated with a mixture of asphalt and creosote, was stocked with oyster seed on May 5, 1970, and was later colonized by *S. unicornis*. ×44.

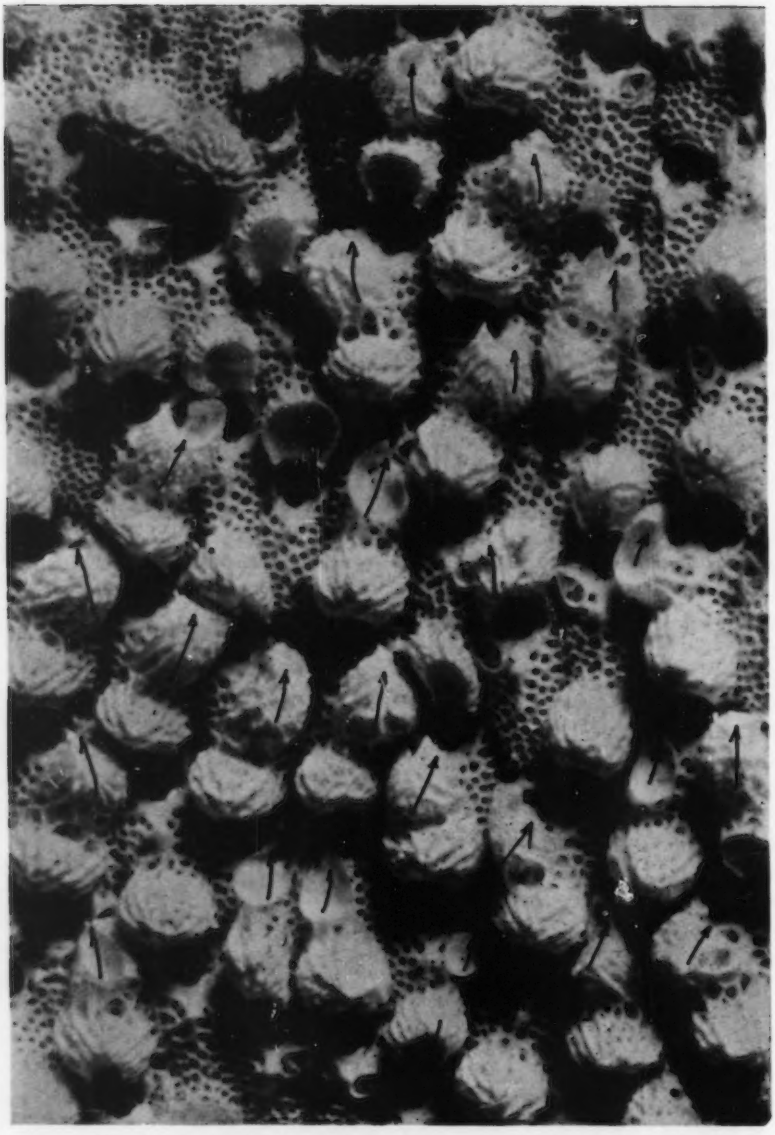

FIG. 3. Hyperplasia developing in colony transferred to Nahcotta boat basin, Willapa Bay; additional ovicells marked by arrows. Colony placed in position on July 29 and removed on August 7, 1970. ×44.

Occurrence of Juvenile and Spawning Atlantic Mackerel in Southeastern Newfoundland Coastal Waters

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PARSONS, L. S., AND V. M. HODDER. 1970. Occurrence of juvenile and spawning Atlantic mackerel in southeastern Newfoundland coastal waters. J. Fish. Res. Bd. Canada 27: 2097–2100.

The capture of juvenile mackerel in Conception Bay, Nfld., during November 1968 represents the first documented record of the occurrence of 0-group mackerel in eastern Newfoundland waters. Their presence suggested the possibility of sporadic mackerel spawning in the area. This was confirmed by the capture of prespawning and spawning mackerel in Placentia Bay during early July 1970.

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ALTHOUGH adult Atlantic mackerel (*Scomber scombrus*) have been taken as far north as Black Island, Labrador (53°46′N) (Parsons, 1970), the southern part of the Gulf of St. Lawrence was presumed to be the northern limit of their spawning range (Templeman, 1966). Most of the spawning of the northern population of mackerel occurs over the Magdalen Shallows in the Gulf of St. Lawrence during June and July (MacKay, MS, 1967). A few mackerel larvae were taken near Cape Anguille in southwestern Newfoundland by the Canadian Fisheries Expedition in its exploration of the Gulf of St. Lawrence during the spring and summer of 1915 (Dannevig, 1918). During the Belle Isle Strait Expedition of 1923, mackerel eggs and fry were taken in August near Cape Breton in the Cape Breton Current, which flows southeastward out of the Gulf of St. Lawrence, but none were taken near western Newfoundland or in the northeastern part of the Gulf (Huntsman et al., 1954). Sette (1943) reported the occurrence of a number of mackerel eggs in a sample of fish eggs collected from Bay of Islands by the Newfoundland Fishery Research Commission and concluded that mackerel spawning takes place occasionally along the west coast of Newfoundland but probably only in bays in which the water warms up to 10 C.

There are no documented records of mackerel spawning in southern or eastern Newfoundland waters. Hind (1877) mentioned that mackerel about 3 inches long appeared about the 20th of July in Notre Dame Bay on the east coast and were numerous there in September and October when adult mackerel were also found, apparently basing his claim on statements by Newfoundland fishermen. However, until the capture of the specimens reported here, reliable capture records of mackerel in southern and eastern Newfoundland and southern Labrador waters appear to have been restricted to post-spawning adults. It has been assumed that the seasonal occurrence of adult

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mackerel in the area represents a postspawning migration probably from the Gulf of St. Lawrence spawning areas. Huntsman et al. (1954) suggested that adult mackerel pass up the west coast of Newfoundland in the warm water and through the Strait of Belle Isle along its south shore. Periodic fluctuations in mackerel abundance in Newfoundland waters have been attributed to variations in surface temperatures with periods of peak abundance being associated with higher than usual surface temperatures (Templeman and Fleming, 1953).

The occurrence of juvenile mackerel in eastern Newfoundland waters is confirmed by the capture of 0-group mackerel in Conception Bay on November 22, 1968. A large body of fish, estimated as covering about 4 square miles, was detected near the surface about 2 miles east of Brigus by the seiner *Marilyn Marie*, which was engaged in exploring for squid (*Illex illecebrosus*) and herring (*Clupea harengus*) under joint sponsorship of the Industrial Development Branch of the Canadian Department of Fisheries and Forestry and the Newfoundland Fisheries Development Authority. Some of the fish were attracted to the surface with mercury lights and a number of specimens were obtained by dipnet. Ten specimens were subsequently brought to the Fisheries Research Board Biological Station at St. John's, Nfld., for examination; 4 were identified as mackerel and the remainder as herring. Fishermen in the area reported that a large mixed school of juvenile herring and mackerel was present in the same general locality for 3 weeks or more prior to the date of capture of these specimens.

The four juvenile mackerel examined by us had total lengths of 93, 104, 111, and 116 mm. Since each of the otoliths had a single opaque zone, the specimens presumably resulted from a spawning during the spring or summer of 1968. Each of the specimens had a vertebral count of 30, which agrees with a mean vertebral count of 30 for both Nova Scotia and Massachusetts specimens (MacKay and Garside, 1969).

There appeared to be two possible explanations for the occurrence of 0-group mackerel in Conception Bay during November 1968. They may have been the progeny of a spawning in southeastern or eastern Newfoundland waters during the late spring or summer of 1968, or they may have been migrants from a known mackerel spawning area, possibly the Gulf of St. Lawrence, a distance of nearly 500 miles from the place of capture via a southern route and 700 miles by a northern route.

Very little is known of the migration and habits of juvenile mackerel. Sette (1950) stated that their distribution early in summer is probably determined by the location of the grounds on which they were spawned and on their subsequent drift from these grounds. He assumed that those that are spawned in the Gulf of St. Lawrence would be found along the shores of the Gulf, presumably mostly along its easterly portions, but published records are lacking. In the known spawning areas juvenile mackerel appear to stay inshore during the summer and fall and even early winter, with catches sometimes being made as late as December. Sette concluded from their distribution

that there is little indication of any extensive migrations before their disappearance in the late fall or early winter. Presumably they then migrate to offshore wintering areas.

In years prior to 1968, for which statistics are available by month and statistical area, catches of adult mackerel were usually first made in August or September in northeastern Newfoundland, presumably from postspawning schools that migrated from the Gulf of St. Lawrence through the Strait of Belle Isle. Subsequently mackerel were taken in most of the bays along the east coast of Newfoundland until November when they presumably migrate southward to wintering areas on the banks off the eastern United States. In 1968 adult mackerel first appeared in southeastern Newfoundland in July with landings being reported from Placentia and St. Mary's bays and on the eastern side of the Avalon Peninsula; no July landings were reported from other eastern or southern Newfoundland areas, although mackerel were taken in most areas from August to November. In 1969 and 1970 adult mackerel appeared in the bays of southeastern Newfoundland during late June and were reported to be abundant in July.

Those mackerel schools, which first appear in southeastern Newfoundland in late June and early July, probably represent an offshoot from the northward migration over the Scotian Shelf into the Gulf of St. Lawrence and spawn after entering Newfoundland coastal waters. A sample of adult mackerel obtained from a codtrap set near Long Harbour, Placentia Bay, on July 3, 1970, contained a high proportion of prespawning and spawning fish. Fishermen in the area reported that schools of adult mackerel were present for several weeks prior to the capture of these specimens. Three of the 200 specimens examined were immature and 11% were maturing (Stage 3) with the gonads occupying about half of the ventral cavity. Fifty-six percent possessed well-developed gonads similar to stages 4, 5, and 6A as defined by Steven (1952). Most of the ovaries contained a large proportion of ripe translucent eggs (average egg diameter 0.75–1.00 mm) and appeared to be very close to spawning. Thirteen percent of the specimens were in actual spawning condition, either running or partly spent. Twenty percent were spent fish, the testes and ovaries being flabby and bloodshot. The male to female sex ratio was 27:73.

These Placentia Bay specimens, which represent the first documented capture of prespawning and spawning mackerel in southern or eastern Newfoundland waters, confirm the occurrence of mackerel spawning in southeastern Newfoundland as suggested by the presence of 0-group mackerel in Conception Bay during November 1968.

Mackerel generally spawn at temperatures from 9.0 to 13.5 C, although eggs have been found in water as cool as 7.3 C and as warm as 17.6 C (Sette, 1943). Temperatures in the warm surface layer in the bays of southern and eastern Newfoundland from mid-July to mid-September generally range from 10 to 13 C at the surface and diminish to 5 C at about 50 m. Although there is considerable year-to-year variation in the temperature pattern and the thickness of the warm surface layer in summer, temperature conditions are

suitable for egg and larval development in some years. The precise time and extent of spawning as well as the degree of survival of the progeny in the Newfoundland area are not yet known and must await more detailed investigations.

The lengths of our juvenile specimens are smaller than previously reported juveniles, captured in late fall (Bigelow and Schroeder, 1953; MacKay, MS, 1967). MacKay (MS, 1967) reported that otoliths from juvenile mackerel, taken in September 1966 from the coastal waters of northeastern United States, with a fork length range of 135–175 mm, showed only an opaque nucleus, and he classed them as 0-group fish. Mackerel taken in July and September 1966 with fork length ranges of 185–265 mm and 245–275 mm, respectively, were classed as age 1-plus. On this basis he concluded that the progeny of spawning in June or July attain a fork length of about 200 mm by the end of the following autumn. Our specimens, captured in late November, were 93–116 mm in total length. This highly significant difference in growth may be the result of a later spawning in the Newfoundland area, development under poorer environmental conditions, or both.

Acknowledgments — We thank Captain Whelan of the vessel *Marilyn Marie* for bringing the occurrence of the juvenile mackerel in Conception Bay to our attention and supplying us with specimens for examination.

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The Case for Accurate Labeling of Superchilled Fish

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GOULD, E. 1970. The case for accurate labeling of superchilled fish. J. Fish. Res. Bd. Canada 27: 2101–2103.

The skeletal muscle of haddock (*Melanogrammus aeglefinus*) contains a malic enzyme activity that can be solubilized by freezing and thawing the flesh but that is not present in the tissue fluid of unfrozen haddock. An objective test, previously used to detect the presence of this normally latent enzyme activity in the tissue fluid of frozen and thawed fish, has been used to discover whether the latent enzyme appears in the tissue fluid of superchilled fish. Enzymograms for superchilled fish are the same as for frozen fish, both showing the latent enzyme activity that is not present in unfrozen fish. The test underscores the need for precise labeling of superchilled fish.

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THE ADVENT of superchilling[1] as a potential commercial method for prolonging the shelf life of fresh fish raises the question whether any fish so treated may accurately be labeled or legally be sold as "fresh."

Although superchilled fish are neither deep-frozen nor stored at 0 F or lower, as is the case with products normally offered as "frozen," nonetheless the superchilling process does partially freeze the flesh, particularly when the process includes an initial crust-freeze, a blast of cold air that lowers the temperature of the outer layers of the fish to approximately 22 F. Gross examination of the flesh of a superchilled fish reveals large ice crystals and gaping myotomes, from which there is appreciable drip when the fish is thawed.

The purpose of this communication is to point out that there exists biochemical proof of cellular disruption brought about by freezing and thawing in superchilled fish. Enzymography (polyacrylamide electrophoresis coupled with a developing medium specific for malic enzyme activity) has already been used to detect whether fish flesh has been frozen and thawed (Gould, 1969); the same procedure can be used to distinguish between fresh fish and superchilled fish. An improved protocol is spelled out in detail in a recent study on shucked oyster meats (Gould and Medler, 1970).

Basis for the test is the differing electrophoretic mobilities of two major forms of malic enzyme (L-malate:NADP oxidoreductase (decarboxylating), EC 1.1.1.40), present in the flesh of most commercial groundfish studied (Gould, 1969). One form is normally soluble (free ME) and is present in the

[1]"Superchilled" is a word brought into use by the fishing industry to describe the condition of a fish stored at several degrees below the freezing point of water (28 F is considered the optimal temperature). It should not be confused with the term "supercooled," which refers to a liquid in metastable equilibrium, and for which a precise chemical definition exists.

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sarcoplasm of unfrozen fish, and the other form (latent ME) must be solubilized by some harsh treatment of the tissue, such as freezing and thawing, homogenization, or bacterial or autolytic spoilage, before it can be detected in the centrifuged whole-tissue fluid (Gould, 1968).

Four requirements for the test should be kept in mind: (1) the polyacrylamide gel formularies should be prepared according to specifications rather than purchased, so that the buffer systems may be made more sharply discontinuous than those in the commercially available formularies; (2) tissue samples should be from the white flesh only. Tissues of the lateral line, more active metabolically, have an earlier and greater rate of autolysis, which can lead to the partial solubilization of latent ME that has been observed in spoiled flesh (E. Gould, unpublished results); (3) the centrifuged tissue fluid should be from whole pieces of white tissue an inch square or more. Homogenization or fine mincing of the tissue releases latent ME. After the fluid has been obtained, however, it may be frozen-stored for later analysis or may be shipped frozen to a laboratory equipped for enzymography; freezing the fluid itself does not affect test results (Gould, 1968); (4) the test is invalid for spoiled fish, where bacterial and autolytic action obscure the picture by gradually solubilizing the latent ME.

The enzymograms in Fig. 1 are from a single catch of fresh haddock, where F represents the fresh fish held 3 days in ice at 38 F; SC′, superchilled fish held at 26 F; SC″, superchilled fish held at 28 F; and X, frozen fish held at −10 F. ME indices[2] for the four tissue fluid samples were 43, 66, 90, and 83, respectively. The heavy band seen in all four gel columns is ascribed to the normally soluble free ME, and the faster-moving band seen in the superchilled and frozen samples is ascribed to latent ME, solubilized by the freeze–thaw process.

The thin, slow-moving band seen in F and SC″ is a thus-far unaccountable variable observed in a few individual haddock specimens. Whenever it has appeared, however, it has been present both in unfrozen and in frozen and thawed samples from the same fish, and is therefore irrelevant to the test here.

There is also a physical method for determining whether fish have been frozen or thawed (Freeze and Makow, 1968), employing ultrasound backscatter. The method calls for relatively expensive instrumentation that may not be within the scope of small laboratories. A more important restriction is the requirement for whole tissue, which limits the test to freshly obtained samples. The Intelectron fish tester, too, which has been tentatively mentioned as a speedy, inexpensive means of determining whether fish have been frozen and thawed, has too stringent requirements for it to be of practical value: the fish must be not only whole but also in excellent condition, "...being neither badly pressed, bruised, damaged, bleached, contaminated, desiccated, heavily parasitized..." (Wittfogel and Schlegel, 1965). Enzymography, on the other

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[2]ME index = the change in absorbance (340 mμ) × 10^3/min per 0.1 ml tissue fluid. Assay conditions have been described elsewhere (Gould, 1965).

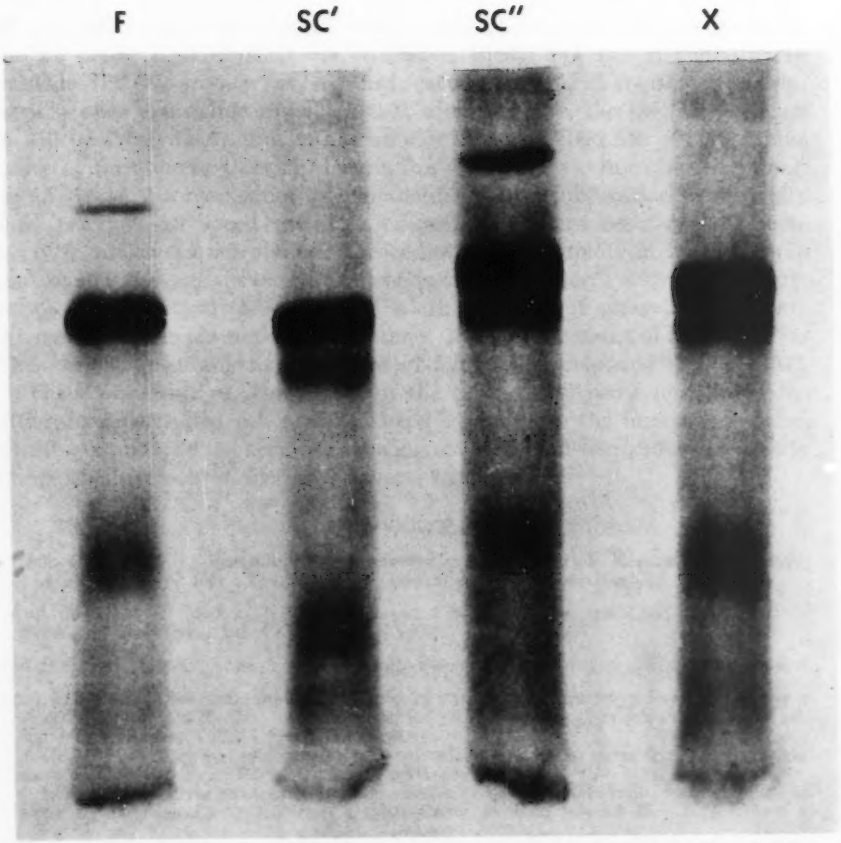

FIG. 1. Photograph of polyacrylamide enzymograms for haddock stored 3 days at different temperatures. F represents malic enzyme activity in the sarcoplasm of fresh fish held in ice at 38 F; SC′, that of superchilled fish held at 26 F; SC″, that of superchilled fish held at 28 F; and X, that of frozen fish held at −10 F. The broad band seen in all four columns is ascribed to free ME and the fast-moving band directly below it in SC′, SC″, and X, to latent ME. The thin, slow-moving band in F and SC″ is an individual variable occasionally seen, and has no bearing on test results.

Gould — J. Fish. Res. Bd. Canada

hand, employs centrifuged whole-tissue fluid, which may be obtained at any time or place and deep-frozen until use without altering test results (Gould, 1968).

There can be no doubt that although it extends the amount of time a trawler may remain at sea without the catch's becoming spoiled, superchilling results in a thawed product that deteriorates more rapidly than normally iced fish (Waterman and Taylor, 1967). Superchilling fish has been said to give the product added shelf life (Gillespie, 1968), and this is undoubtedly true while the fish are kept superchilled; certainly bacterial spoilage is slowed. But it is only reasonable to expect that after thawing, the shelf life of such fish will be shorter than that of unfrozen or unsuperchilled fish of comparable quality at the time of thawing. Disruption of cellular structure in frozen and thawed tissue, disorganization of metabolites, and solubilization of normally bound enzymes all speed the pace of postmortem catabolic events. Some glycolytic and nucleotide changes proceed even more rapidly at 26–28 F than at customary iced-storage temperatures (Nowlan and Dyer, 1969). Temperature control, too, is critical (Merritt, 1965; Carlson, 1969); if superchilled fish are held even 1 degree lower than the optimal 28 F, the amount of ice formation makes the fish unsuitable for filleting or smoking (Waterman and Taylor, 1967).

These observations, together with the biochemical proof of cellular disruption in superchilled fish reported here, all point to the importance of establishing standards for accurate labeling. Superchilled fish should be clearly distinguished from fresh fish in the marketplace.

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Meristics and Morphometrics of Sand Launce in the Newfoundland Area

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Analyses of meristic and morphometric data of sand launce in the Newfoundland area revealed that the Grand Bank and St. Pierre Bank specimens were slender with relatively short heads and high meristic counts whereas those from Trinity Bay and St. Mary's Bay were deep-bodied with relatively longer heads and low meristic counts. Consequently, offshore launce were provisionally assigned to *Ammodytes dubius* and inshore specimens as *Ammodytes hexapterus*. Differences in body form and meristics were also evident within the two groups.

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SAND LAUNCE (*Ammodytes* spp.) are very common in Newfoundland coastal waters and offshore banks, but very little published information is available on their species composition. Dannevig (1918) and Frost (1938), who provided some information on the distribution and seasonal abundance of launce larvae and postlarvae in the Newfoundland area, apparently assigned species names on the basis of occurrence rather than taxonomy. Dannevig (1918) assigned his specimens to *A. tobianus* (Linnaeus), which is an inshore species found only in European waters. Frost (1938), on the other hand, assigned her specimens to *A. americanus* (DeKay) (= *A. hexapterus* Pallas, 1831), which is the inshore species of launce in the northwest Atlantic although many of the launce larvae in her data were caught offshore on the Grand Bank and St. Pierre Bank. The only meristic data published on launce from Newfoundland waters is by Richards et al. (1963) (10 specimens from the Grand Bank assigned to *A. dubius* (Reinhardt, 1838) and 9 specimens from Lake Melville, Labrador, assigned to *A. hexapterus*) and Backus (1957) (26 specimens from Labrador, 13 assigned to *A. americanus* and 13 assigned to *A. dubius*).

Considerable numbers of sand launce have been collected by research vessels of the St. John's Biological Station of the Fisheries Research Board of Canada incidental to catches of other fishes and a large amount of data has been collected on their meristic and morphometric characters. These meristic and morphometric characters form the basis of this paper. For species identification the taxonomic groupings of Richards et al. (1963) for North American *Ammodytes* are used, i.e., there are considered to be two morphological types provisionally separated into species: *A. dubius*, the offshore form, and *A. hexapterus*, the inshore form.

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Materials and methods — Offshore specimens of sand launce from various localities of the Grand Bank and St. Pierre Bank (Fig. 1) were caught by the research vessels *Investigator II* and *A.T. Cameron* using a No. 36 or No. 41.5 bottom trawl with a codend liner of mesh size ranging from 15 to 20 mm. These launce consisted of maturing and ripe adults ranging in total

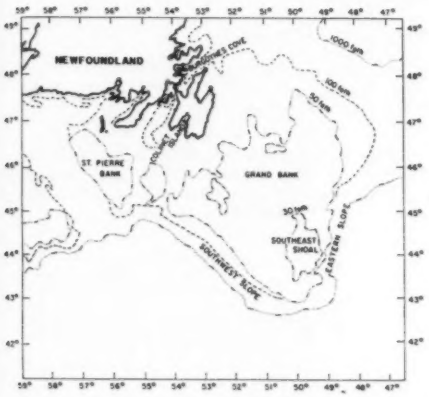

FIG. 1. Localities and place names mentioned in the text.

length from 150 to 300 mm (Table 1). Inshore specimens of launce from Burgoynes Cove in Trinity Bay and Colinet Island in St. Mary's Bay (Fig. 1) were caught by a small-meshed beach seine. The specimens from Burgoynes Cove were ripe adults ranging in total length from 200 to 240 mm whereas the Colinet Island sample consisted of juvenile launce of 60–120 mm. The launce were examined in detail after preservation in 50% isopropyl alcohol.

TABLE 1. Origins of samples on which this report is based. The maturity stages used are from Maçer (1966).

Date	Locality	No. samples	No. fish	Maturity stage	Length range (*mm*)
May 20, 1955	Southwest slope, Grand Bank	1	150	2	200–300
June 3, 1956	St. Pierre Bank	1	109	2	200–290
Sept. 12, 1961	Colinet Island, St. Mary's Bay	1	44	1	60–120
Sept. 28, 1961	Burgoynes Cove, Trinity Bay	1	29	3	200–240
July 10–15, 1968	Southeast Shoal, Grand Bank	5	349	2	150–275
Oct. 4, 1968	East slope, Grand Bank	1	185	3	195–280

Vertebrae (866 specimens) and anal (641 specimens) and dorsal (591 specimens) fin rays were counted from radiographs. Fin ray counts were checked by direct counts. The hypural complex was excluded from all vertebral counts. Body depths were measured just in front of the dorsal fin. Head length was measured (point to point with calipers) from the tip of the mandible to the most posterior edge of the operculum, i.e., greatest head length. Standard length was measured from tip of mandible to base of the caudal fin. All morphometric measurements were made by the author and recorded to the nearest half-millimeter.

Meristics — Vertebral numbers of launce from the eastern and southwestern slopes of the Grand Bank and from St. Pierre Bank (Fig. 2) ranged

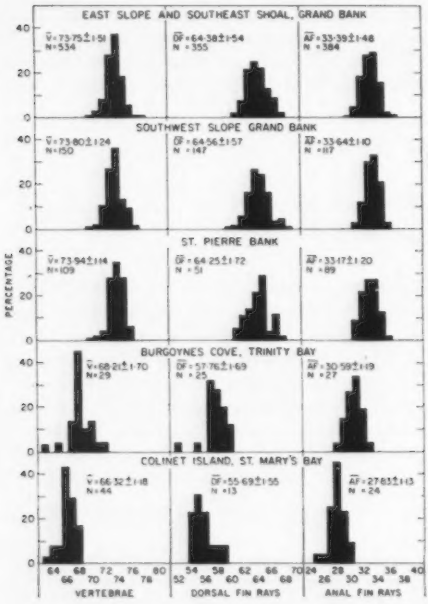

FIG. 2. Meristic counts of *Ammodytes* examined in the Newfoundland area together with mean counts plus or minus one standard deviation of mean.

from 70 to 78 with a single mode at 73. Dorsal fin ray counts of specimens from these areas ranged from 60 to 69 with modes at 64, 64, and 65, respectively. Anal fin ray counts ranged from 30 to 37 with modes at 34 for all three areas.

Meristic counts of inshore samples of launce, from Burgoynes Cove and Colinet Island, were considerably lower than those of offshore samples, with vertebral counts ranging from 63 to 72, dorsal fin rays from 52 to 60, and anal fin rays from 25 to 33.

t-Tests at $P < 0.01$ were applied to the differences between the means of the meristic characters in the offshore and inshore samples. In the offshore group a significant difference in mean anal fin ray numbers was found between the southwest slope of the Grand Bank and St. Pierre Bank samples. In the inshore group significant differences were found between the Burgoynes Cove sample and the Colinet Island sample in all meristic characters.

Morphometrics — Log–log regressions of body depth and of head length on standard length were calculated for the southwest Grand Bank, St. Pierre Bank, and Burgoynes Cove specimens and the lines obtained (Fig. 3) were compared by analyses of covariance. There was no significant difference between the two offshore samples of launce in the slopes of the lines of body depth on standard length ($F = 0.46$ for $F_{.01} = 6.76$) or of head length on standard length ($F = 0.03$ for $F_{.01} = 6.76$) although differences in the adjusted means were significant ($F = 408.10$ for body depth; $F = 97.42$ for head length).

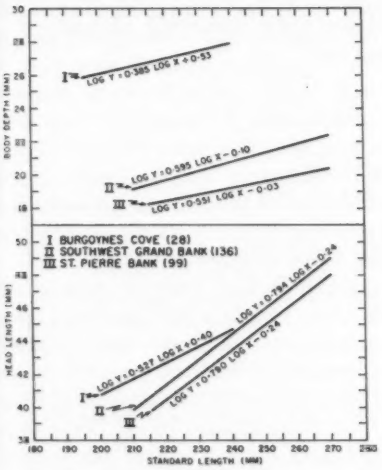
I BURGOYNES COVE (28)
II SOUTHWEST GRAND BANK (136)
III ST. PIERRE BANK (99)
LOG Y = 0·585 LOG X + 0·53
LOG Y = 0·595 LOG X − 0·10
LOG Y = 0·551 LOG X − 0·03
LOG Y = 0·537 LOG X + 0·40
LOG Y = 0·784 LOG X − 0·24
LOG Y = 0·780 LOG X − 0·24
BODY DEPTH (MM)
HEAD LENGTH (MM)
STANDARD LENGTH (MM)

FIG. 3. Log–log regression of body depth and head length on the standard length of *Ammodytes* from the Newfoundland area.

The regression of body depth on standard length for the sample from Burgoynes Cove was not significantly different in slope from that for the offshore samples ($F = 1.64$ for $F_{.01} = 6.76$) although the difference in the adjusted means was significant ($F = 1834.51$). The regressions of head length on standard length for the inshore and offshore samples were significantly different in slope ($F = 19.39$).

Body depth as a percentage of standard length ranged from 6.1 to 10.5% with a mean at 8.3% in the offshore material and from 8.2 to 14.4% with a mean at 12.3% in the inshore material. Although the mean percentage for body depth of the offshore specimens is well within the range reported for offshore launce by Richards et al. (1963) for the northwest Atlantic area, the corresponding value for the inshore specimens is considerably higher than the range of 7.1–9.7% recorded by these authors for the inshore launce. This difference could be due to differences in maturity stages between Richards' and the present data.

Head length as a percentage of standard length ranged from 16.6 to 20.1% with a mean at 18.3% for the offshore samples; the specimens of inshore launce ranged from 17.9 to 20.9% with a mean at 19.2%. These ranges are very similar to those reported by Richards et al. (1963).

Discussion and conclusion — The differences in meristic characters and morphometrics between the offshore and inshore launce in the Newfoundland area correspond to the basic differences between *A. dubius* and *A. hexapterus* as described by Richards et al. (1963), i.e., *A. dubius* is a slender launce with high meristic counts whereas *A. hexapterus* is a deep-bodied launce with low meristic counts. Consequently the Grand Bank and St. Pierre Bank sand launce were provisionally assigned to *A. dubius* whereas the Burgoynes Cove and Colinet

Island specimens were provisionally assigned to *A. hexapterus*. Significant differences in body form and meristics within the *A. dubius* and *A. hexapterus* groups respectively were also shown to exist in the Newfoundland area and these may be of use in future population studies. Richards (1965) states that the meristic and morphometric differences between *A. dubius* and *A. hexapterus* that were previously used as criteria for species separation are apparently not genetically determined but have been induced by environmental factors, particularly temperature and salinity. Apparently, the colder, more saline water offshore and to the north slows development and produces thin launce with higher meristic counts than those from the warmer, less saline inshore waters.

In the northwest Atlantic there is a marked inshore–offshore cline in the meristic counts of *A. hexapterus* (Richards et al., 1963) with specimens from semienclosed bays having low meristic counts whereas those from exposed coastal areas have a wide range of meristic counts. Trinity Bay meristic counts correspond to counts of *A. hexapterus* from along exposed coastal areas whereas St. Mary's Bay counts fit into the group from semienclosed bays. These bays are not semienclosed and the Newfoundland data do not fit into the general pattern.

Richards et al. (1963) state that there is a general trend of increasing meristic numbers with increasing latitudes in *A. dubius*. Newfoundland material is anomalous as it had mean vertebral counts lower than those from Nova Scotia (Scott, 1968) yet higher than those from Labrador (Backus, 1957). Clark and Vladykov (1960) found that the vertebral counts of haddock from St. Pierre Bank and Georges Bank were lower than those from the Nova Scotia banks and correlated this with the lower spawning temperatures on the Nova Scotia banks. A similar explanation may account for the apparent anomaly in the vertebral counts of launce from the Newfoundland area.

Acknowledgments — The author gratefully acknowledgments the assistance of Mr A. M. Fleming who provided the samples of launce from Burgoynes Cove and Colinet Island.

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Muscle Protein Electrophoresis in the Genus *Salmo* of Eastern Canada

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GRAY, R. W., AND J. A. MCKENZIE. 1970. Muscle protein electrophoresis in the genus *Salmo* of eastern Canada. J. Fish. Res. Bd. Canada 27: 2109–2112.

The muscle protein pattern of Atlantic salmon (*Salmo salar* L.) examined differed markedly from that of brown trout (*Salmo trutta* L.) and rainbow trout (*Salmo gairdneri* Richardson). Atlantic salmon muscle proteins from six areas were not influenced by the differences in sex, stage of maturation, or geographical location. The muscle proteins of brown trout taken from two areas in Newfoundland were similar and could not be easily distinguished from that of rainbow trout from Newfoundland. A polymorphism was observed in the pattern of rainbow trout from Great Pond, Nfld. The consistency of the patterns within species suggests that they may provide useful characters for identification purposes.

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THE ELECTROPHORETIC PATTERNS of the muscle proteins of several species of the genus *Salmo* have previously been described (Tsuyuki and Roberts, 1963, 1965; Tsuyuki et al., 1965; Nyman, 1967; Haen and O'Rourke, 1968); however, in many of these studies the number, size, stage of maturation, sex, and origin of the specimens were not given and under such conditions one cannot be certain that the patterns as described were indeed characteristic of the species (Rasmussen, 1969).

This is a report on electrophoretic patterns of the muscle proteins of adults of brown trout (*Salmo trutta* L.), rainbow trout (*Salmo gairdneri* Richardson), and landlocked Atlantic salmon (*Salmo salar* L.), each from two geographically isolated populations in eastern Canada, and of various stages of anadromous Atlantic salmon from three river systems in New Brunswick and waters off Greenland.

Materials and methods — Landlocked and anadromous forms of Atlantic salmon were collected from six geographically isolated areas (Table 1). Although the three river systems in New Brunswick are geographically isolated from each other, mixing of salmon stocks may have occurred not only through natural straying but also through past stocking practices. Brown trout and rainbow trout were each collected from two geographically isolated populations in Newfoundland. Populations of each of these two species are of different genetic origins and in the case of brown trout show some minor morphological and color variations (Scott and Crossman, 1964; Andrews, 1965).

Immediately after capture, fish were decapitated, packed in ice, and transported to deep-freeze facilities. Crude extracts of skeletal muscle were prepared by homogenizing 1 g of muscle with 1 ml of distilled water in an ice-water bath. The homogenates were allowed to stand for 1 hr at 4 C and were centrifuged at 12,000 *g* for 20 min. The supernatants were used immediately for electrophoresis.

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TABLE 1. Data on the collections of fish used in muscle protein electrophoresis in the genus *Salmo*.

Species	Location	Date of capture (1969)	Fork length (*cm*)	Stage of maturation	No. examined
Salmo trutta	Forest Pond, Nfld.	May 17	27.0–28.9	Adult	15
	Long Pond., Nfld.	"	25.2–31.7	"	21
Salmo gairdneri	Murray's Pond, Nfld.	"	22.5–28.4	"	11
	Great Pond, Nfld.	"	17.1–24.7	"	6
Salmo salar					
Landlocked forms	Forest Pond, Nfld.	"	19.4–24.5	"	32
	Chamcook L., Nfld.	May 4	33.6–43.2	"	6
Anadromous forms	Restigouche R., N.B.	June 14		"	3
		June 13	13.2–16.3	Smolt	3
		Nov. 1	62.1–121.3	Adult	20
	Miramichi R., N.B.	May 21	58.8–71.8	Adult	2
		May 21	54.2–56.7	Grilse	6
		May 25	12.1–16.4	Smolt	2
		May 28	65.6–84.1	Adult	41
		June 26		Grilse	19
		July 4	50.1–55.1	"	33
		Aug. 14		"	8
		Aug. 21		"	2
	Saint John R., N.B.	July 10	71.4–93.2	Adult	5
		July 10	52.3–55.3	Grilse	4
	Greenland	June		Adult	5

Vertical starch-gel electrophoresis (Smithies, 1955) was carried out as modified by Boyer and Hiner (1963). The vessel buffer consisted of 0.2 M tris (hydroxymethyl) amino methane (Tris) and 0.2 M HCl, adjusted to *p*H 8.6 (Gomori, 1955). The gel buffer consisted of 150 ml of 0.2 M Tris–HCl buffer, *p*H 8.6, and 450 ml of distilled water. The starch (Connaught Medical Laboratories, University of Toronto) concentration was 13–14%. Electrophoresis was carried out for 9 hr at 4 C, and at 20 ma and 450–470 v. Gels were sliced and stained for total protein with 0.2% Amido-black 10B (E. Merck, A. G. Darmstadt, Germany). The gels were destained in a 5:5:1 mixture of water, methanol, and acetic acid.

Bands were numbered in increasing order of mobility from the origin to the anode (A1-A12); the three bands on the cathodic side at this *p*H were labelled C1–C3. Minor variations in the intensity and presence or absence of one or more of bands A1–A4 were observed in some instances; if these differences could not be duplicated in the same gel consistently, they were not considered as valid differences.

Results and discussion — Analysis of the muscle protein patterns indicated differences between the three species whereas Haen and O'Rourke (1968), using polyacrylamide gel rather than starch-gel electrophoresis, were unable to distinguish between the muscle proteins of brown trout and Atlantic salmon. Atlantic salmon muscle proteins differed markedly from those of brown trout and rainbow trout (Fig. 1). Most notable was the absence of band A5 in Atlantic salmon; differences in the intensity of band A9 were of less significance. Although the muscle myogen pattern of brown trout was similar to that of rainbow trout, it was not identical. Band A7 was consistently more diffuse, and band

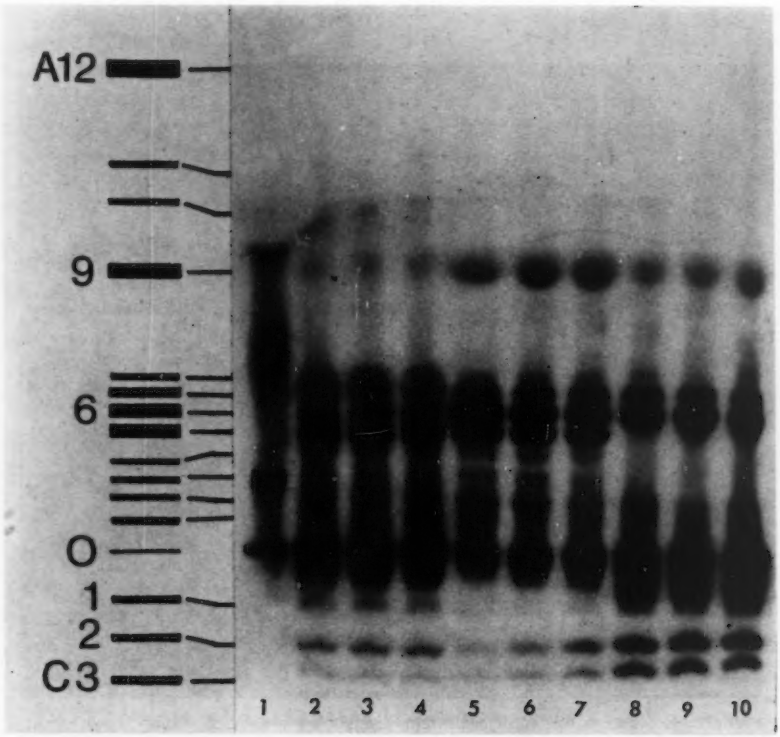

FIG. 1. Muscle protein electropherograms of randomly selected adult representatives of each species of the genus *Salmo* found in eastern Canada. Slot 1, bovine serum; slots 2–4, rainbow trout; slots 5–7, brown trout; and slots 8–10, Atlantic salmon. Fish in slots 3, 5, and 8 are males.

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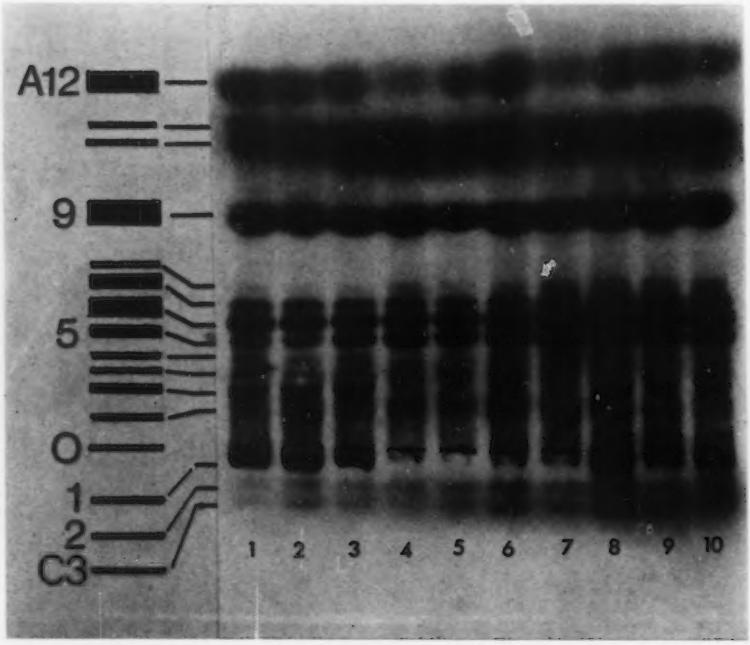

FIG. 2. Representative muscle protein electropherograms of adult brown trout from Forest Pond (slots 1–3) and Long Pond (slots 4–10), Nfld. Fish in slots 2, 3, 7, and 8 are males.

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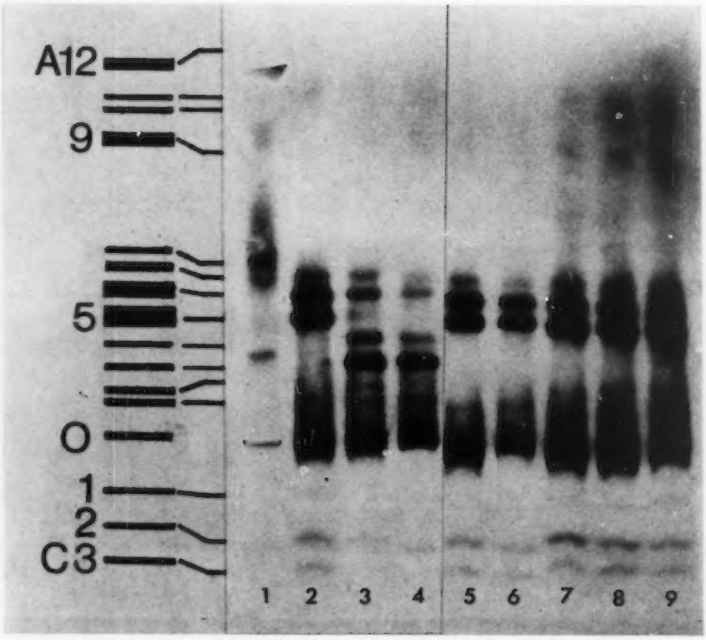

FIG. 3. Representative muscle protein electropherograms of adult rainbow trout from Great Pond (slots 2–4) and Murray's Pond (slots 5–9), Nfld., and of bovine serum (slot 1). All fish are female except that in slot 9.

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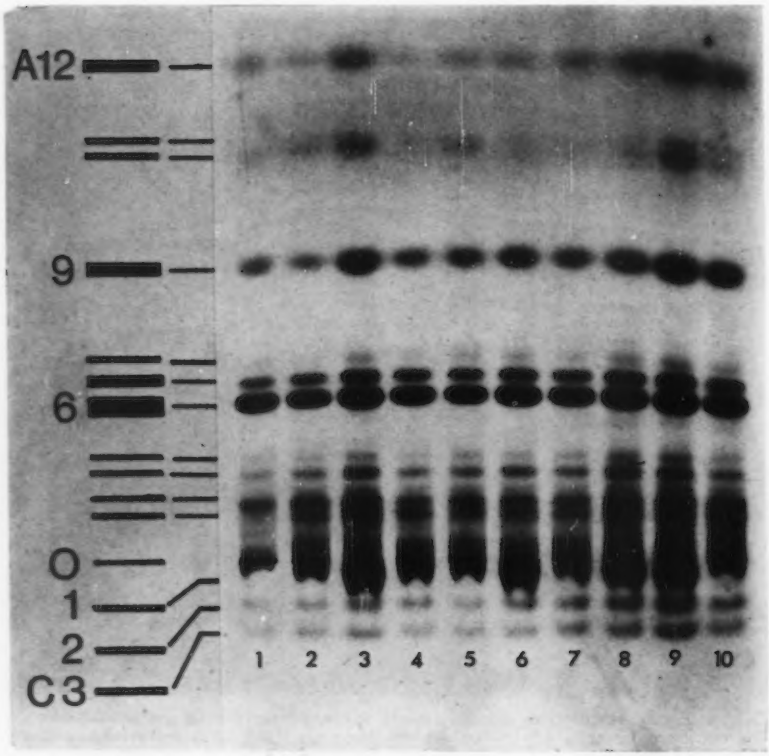

FIG. 4. Representative muscle protein electropherograms of Atlantic salmon from Forest Pond (slots 1–2), Chamcook Lake (slot 3), Restigouche River (slots 4–5), Saint John River (slots 6–7), Miramichi River (slots 8–9), and Greenland (slot 10). Specimens in slots 1–3, 5, and 6 are males. All fish are adults except that in slot 4, which is a smolt.

A9 was weaker, in rainbow trout than in brown trout. Despite these differences, distinguishing brown trout from rainbow trout solely on the basis of their muscle protein pattern was difficult.

The muscle protein patterns of the two populations of brown trout from Newfoundland were identical (Fig. 2). The differences in sex and geographical location of the fish did not affect the muscle myogen pattern.

The muscle protein patterns of the rainbow trout adults from Murray's Pond were similar for males and females (Fig. 3). However, among adult rainbow trout from Great Pond, two specimens out of six examined were different from the pattern shown by fish from Murray's Pond. This polymorphism is shown in Fig. 3.

The muscle protein pattern of Atlantic salmon (Fig. 4) was not influenced by the differences in sex, stage of maturation, or geographical location. Anadromous fish in various stages of maturation from the three river systems in New Brunswick and from Greenland had patterns similar to those of landlocked fish from Forest Pond, Nfld., and Chamcook Lake, N.B. The early and late migratory runs of Atlantic salmon in the Miramichi River (Saunders, 1967) had identical muscle proteins.

Although Nyman (1967) reported polymorphisms in the muscle proteins of Atlantic salmon from Gaspé, Canada, none were found in this study. The variations that he described appear to correspond to minor bands between those labelled A8 and A9. These minor bands when observed in our study appeared in all specimens of a particular electrophoretic run, regardless of population, but could not be consistently duplicated. In a single salmon from Forest Pond, band A2 was missing (Fig. 4). Since this was the case in only one fish out of total of 191 salmon examined, this was considered a rare variant.

The consistency of the muscle protein patterns in each species described here despite the differences in sex, stage of maturation, and geographical location of the fish suggests that these patterns may provide useful characters in the identification of the species of the genus *Salmo*.

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Reproductive Cycle, Size at Maturity, and Sexual Composition of Commercially Harvested Weathervane Scallops (*Patinopecten caurinus*) in Alaska

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Findings of this study indicated that scallops spawn in the summer, primarily during early June, and also through mid-July. Spawning is complete, and only one annual spawning occurs. Sexual composition of mature scallops appeared as a 50:50 ratio. Most scallops were mature when three or more concentric rings (annual rings) were present on the upper valve and all after four or more were present. Scallops off Yakutat grow slower and are smaller at maturity than scallops off Kodiak Island. Growth and size differences did not seem directly related to time of maturity.

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EXPLORATIONS FOR SCALLOPS of commercial size and quantity in Alaska began in late 1967. These efforts were successful and by the end of 1969 more than 3.7 million lb of scallop meats were landed at Alaskan ports.

Objectives of this study were to determine: (1) size at maturity with reference to the number of concentric, or annual, growth rings present on the upper valve; (2) reproductive cycle and spawning period; and (3) sex ratio of commercially caught scallops in Alaska. Prior to this study little information existed on Alaska scallop stocks and knowledge on the above objectives is indispensable for the proper management of this resource.

Methods and procedures — Data for this study were collected from August 1968 through December 1969 aboard commercial scallop vessels. Samples were obtained between Cape St. Elias and Cape Fairweather in the Yakutat region, and in the vicinity of Kodiak Island, especially from the Marmot Bay–Portlock Bank region (Fig. 1).

FIG. 1. Alaska, showing major area of commercial scallop fishing.

Random sampling was accomplished by obtaining a shovel-full of about 25 individuals from the catch prior to any sorting or processing. It was assumed that scallops in the catch were not stratified by size. The normal procedure of upending the dredge to dump out the catch resulted in further mixing. Six or more samples were taken during each fishing day.

Samples of live scallops were also obtained from scallop vessels at the return of a fishing trip. The scallops were placed in a plastic container and supplied with a fresh supply of sea water. In this manner scallops could be kept alive for at least 3 days.

As soon as the sample was taken, the scallops were shucked and the gonads were examined macroscopically for determination of sexual maturity. Live samples brought ashore for laboratory study were handled in an identical manner except that all scallops with two, three or, four concentric rings were measured to the nearest millimeter in a straight-line distance from umbo to outer shell margin.

External features of gonad development — The stages of maturation were defined through macroscopic examination. It was also possible to determine sex through color comparisons of ovaries and testes. The definitions are as follows:

(I) Empty or spawned out: Gonad reduced in size and collapsed, contains free water throughout, transparent, loop of alimentary canal clearly visible. Testes nearly colorless; ovaries a dull amber to nearly colorless.

(II) Initial recovery: Gonad increasing in size, contains one-quarter to one-half the estimated capacity of sex products, free water exists in portions, loop of alimentary canal visible but fading.

Portion of testes containing sex cells cloudy white, rest transparent; ovaries amber to dull pink or orange.

(III) Filling: Gonad near maximum size, contains three-quarters or more of the estimated capacity of sex products, free water exists only as small canals or at the distal end of the gonad, loop of alimentary canal visible only where it is close to the surface. Testes pasty white; ovaries orange to bright orange.

(IV) Full or ripe: Gonad relatively large in relation to other body parts, completely full and rounded, contains no free water, loop of alimentary canal not usually visible, texture appears granular. Testes flat white; ovaries bright orange.

(V) Immature or juvenile: Gonad relatively small in relation to other body parts, angular and flattened, transparent and colorless.

The gonads of 17,971 sexually mature scallops were macroscopically examined during this study. Depending upon the external features each gonad was placed into one of the four stages of sexual development. In practice it proved difficult to clearly differentiate through external examinations between the termination of one stage and the beginning of another. The rate of recovery after spawning varied from one area to another, and in some instances within the same area. The problem of overlapping between stages was further compounded because the stage of development was visually estimated and several observers were employed during the study period. These factors were not considered critical, however, as macroscopic differences between unspawned individuals (stage IV), and recently spent individuals (stage I) were clear and unmistakable. In addition an incompletely spawned individual could readily be detected from an individual that had completed spawning.

Initially, the criteria for separation of the stages of maturation were not well defined, but external appearance, fullness, and coloration were noted. Descriptions were based upon laboratory observations with reference to Mason (1958) for *Pecten maximus* and to Sastry (1963) for *Aequipecten irradians*.

Reproductive cycle — Scallop gonads during August 1968 were empty, watery, transparent, and nearly colorless (stage I), although it is likely that closer examination would have revealed a portion of them in the early recovery stage. No samples were taken during September and October of 1968, but beginning in November of 1968 and extending through December of 1969, one to five samples were examined monthly. Gonad samples observed during November and December of 1968 from the Kodiak area indicated they were at least three-quarters full (stage III) and contained some free water. Observations in the latter part of January 1969 in the Cape Karluk area of Kodiak indicated the gonads were full or ripe (stage IV) and no free water was visible. Hence, it is evident that the division point between stages III and IV occurred between mid-December and the latter part of January (Tables 1 and 2). The gonads remained in the full or ripe condition in all areas for several months, although there appeared to be a progressive enlargement and rounding of the gonad, and coloration became more distinct with the passing of time.

Prior to May 19 in the Yakutat area and June 3 in the Kodiak area, no spawned or partially spawned individuals were found in any of the samples examined. On June 8 in the Kodiak area and June 16 in the Yakutat area samples were examined that clearly indicated spawning had occurred in all individuals examined, and in each instance spawning was complete (Fig. 2).

Apparently, spawning began after May 19 in the Yakutat area and was completed prior to June 16. However, this statement should be qualified since samples from the Yakutat area were relatively small, with extended

TABLE 1. Observations on the stages of gonad maturity and sexual composition of scallops from the Yakutat area, 1968–69.

Date		Collection location	Sample size	Male	Female	Stage of gonad condition I	II	III	IV	V[b]
1968										
Aug.	4–8	Dry Bay	4348[a]	–	–[b]	4348				
1969										
Feb.	17	Yakutat Bay	99	53	46				99	
	25	Cape Fairweather	1402[a]	657	745				1402	
Mar.	8	Cape Yakutaga	100	40	60				100	
	16–19	Icy Bay	1361[a]	701	660				1361	
	27	Icy Bay	168	79	89				168	
Apr.	1	Cape Yakutaga	78	39	39				78	
May	19	Cape Yakutaga	60	20	36				56	4
June	16	Icy Bay	80	46	34	80				
July	21	Cape Yakutaga	70	52	18	21	45	4		
	26	Cape Yakutaga	52[a]	32	19	51				1
Aug.	13	Dry Bay	943[a]	–	–	928	12	3		
Total			8761	1719	1746	5428	57	7	3264	5

[a]Samples processed at sea.
[b]Samples not sexed or immature.

periods between sampling. By and large, however, it is my opinion that spawning began and ended in the Yakutat area between the dates indicated. In the Kodiak area, samples were larger, periods between samples shorter, and, in general, samples were more representative of the fishing areas. The data suggest that the majority of scallops spawned between June 3 and June 8. However, 21 of 250 scallops examined between July 12 and 14 were only partially spawned. The gonads of the 21 partially or incompletely spawned individuals were largely empty or spent, but small clusters of free eggs and sperm could be seen and the gonads did not appear entirely collapsed.

Samples examined in early August indicated initial recovery was well under way, and by the end of August the majority of gonads were sufficiently recovered so that gametes were visible and sex was easily determined by color. Recovery progressed steadily thereafter, and by late November the cycle was complete, as the gonads were again at least three-quarters full of sex products.

Spawning scallops in this study were observed only during June and early July, and only one annual spawning occurred. It is likely that timing of the spawning period will exhibit yearly fluctuations, or even that seasonal variation occurs between one area and another. However, I think it is conclusive that they spawn during the summer, primarily during early June, but extending into July. The mechanism that triggers spawning is uncertain, but temperature changes seem to be involved. In laboratory experiments on sea scallops from the east coast it was found that they could be induced to spawn by first slowly

TABLE 2. Observations on the stages of the maturity and sexual composition of scallops from the Kodiak area, 1968–69.

Date		Collection location	Sample size	Male	Female	Stage of gonad condition I	II	III	IV	V[b]
1968										
Nov.	22	Cape Karluk	120	53	60			113		7
"	29-Dec. 12	Cape Karluk	1539[a]	–	–[b]			1539		
Dec.	12	Halibut Bay	120	65	52			117		3
1969										
Jan.	29	Cape Karluk	116	61	55				116	
Feb.	17	Malina Bay	100	51	48				99	1
	28	Cape Chiniak	454[a]	243	211				454	
Mar.	3–11	Marmot Island	230[a]	132	96				228	2
	12	Marmot Island	59	35	23				58	1
Apr.	7	Cape Ikolik	79[a]	37	42				79	
	11	Ugak Island	550[a]	257	293				550	
	9–21	Marmot Island	503[a]	247	256				503	
	21	Ugak Island	108	63	40				103	5
May	8–17	Halibut Bay	825[a]	396	421				817	8
	23	Katmai Bay	75[a]	33	42				75	
	28–31	Kukak Bay	430[a]	218	212				430	
June	3	Kupreamof Strait	172[a]	75	97				172	
	8	Marmot Bay	75	48	27	75				
July	12	Cape Ikolik	125	86	37	116			7[c]	2
	14	Ugak Island	125	67	55	108			14[c]	3
	30-Aug. 8	Marmot Bay	1529[a]	836	680	1073	443			13
Aug.	11	"	100	47	53	49	51			
	22	"	44	17	27	8	36			
	30–31	"	350[a]	210	139	50	298	1		1
Sept.	3–11	"	596[a]	340	255	42	553			1
	12	"	52	20	32	14	19	19		
Oct.	28	"	75	33	42		75			
Nov.	21–25	"	534[a]	278	256			534		
	30	"	100	48	52			100		
Dec.	17	"	80					76	1	3
Totals			9265	3996	3603	1535	1475	2499	3706	50

[a]Samples processed at sea.
[b]Samples not sexed or immature.
[c]Individuals partially spawned.

raising and then sharply dropping water temperatures (Posgay, 1950, 1953). Sea scallops on portions of the Georges Bank area have been observed spawning during late September and October, and of those examined, 92% spawned or were in the process of doing so within a 4-day period (Posgay, 1958).

Sexual composition — The earliest published data on observations of weathervane scallop gonads in Alaska (Haynes and Powell, 1968) were made

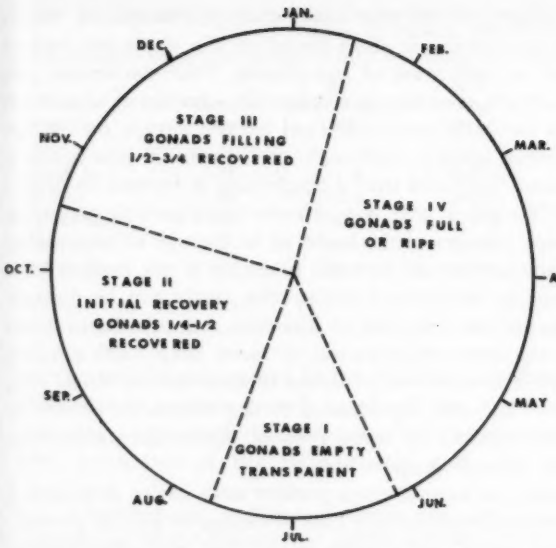

FIG. 2. Reproductive cycle of the weathervane scallop in Alaska. Considerable overlap occurs between each stage of development. Divisional lines are based on observations that a significant proportion of the individuals had reached that stage of maturation by the dates indicated.

during January 1968 in the vicinity of Kodiak Island, Alaska. They state only that the gonads were "ripe" indicating that spawning had not recently taken place.

It is clear that weathervane scallops are dioecious since no hermaphroditic individuals were reported. Also, the sexes of the mature adult scallop are easily distinguished by the characteristic white coloration of the testes and bright orange of the ovaries. The gonad of the adult scallop is full or ripe (stage IV) from about mid-January through the end of May, or until spawning begins. During this period 7087 individuals were sexed: 3531 were males (49.8%), and 3556 were females (50.2%), nearly a 1:1 ratio. Sex determination at other times of the year, especially during the spawning and postspawning period (stage I) was more difficult. At this time the gonads of both sexes appeared similar, although the ovaries of the females tended to retain a faint amber or light pink coloration. In the laboratory, sex could be determined microscopically at any time of the year but for field purposes this technique was considered unnecessary and impractical.

Size at maturity — It is beyond the scope of this paper to enter into a lengthy discussion of the size and growth rates of commercially harvested weathervane scallops in Alaska. However, to state in meaningful terms when a scallop had reached sexual maturity, it became necessary to classify them into size groups according to the number of concentric rings present on the upper valve. This was done only for shells having two, three, and four rings present. Shell heights were determined by measuring, to the nearest millimeter, the straight-line distance from umbo to outer shell margin. This measurement makes no allowances for differential seasonal growth.

Studies on age and growth of the east coast scallop, *Placopecten magellanicus*, have shown that the concentric rings found on the shells are formed annually and are therefore an indication of age (Baird, 1953; Stevenson and Dickie, 1954). During this study monthly or bi-monthly samples of about 100 shells of the smallest sizes available were collected by the author for an age and growth study by another agency. Although the results of this research are unpublished, observations indicated that a single ring is formed annually; it begins to appear by late December, with new or more rapid growth appearing by late April or May. There remains some doubt as to the age of the scallop at the time the first ring has completely formed; hence, it is not implied that the number of rings present as determined during this study can be directly related to the true age of the animal. I do believe, however, that my observations on the formation of the ring from examination of more than 1200 samples gathered over a 1-year period is sufficient evidence to state that a single ring is in fact formed annually. This adds significance to the discussion of size at maturity in relation to the number of rings present. Eventual publication of age and growth studies should support this.

The number of concentric or annual rings present were easily determined since young scallop shells were comparatively free of excessive marine growth. Apparently, so-called "false rings" or checks occur, but my observations tend to indicate these are normally distinct from the annual growth rings, at least after the formation of the initial growth ring.

Examination of 2027 individual scallops during the course of this study showed that 85 had 4 annual rings, 115 had 3 annual rings, and only 9 had 2 annual rings present. No scallops with less than two annual rings present were recovered from the samples examined. The reason for the absence of smaller scallops in the commercial catch is unknown but may be due to gear selectivity, or stock segregation, or both.

Scallops with three annual rings present from the Kodiak area (sample size = 93) ranged from 74 to 128 mm in shell height, with an average of 104.2 mm. Those from the Yakutat area (sample size = 22) ranged from 73 to 92 mm in shell height, with an average of 84.6 mm. Only nine scallops with two annual rings present were found in the Kodiak samples, none from Yakutat. Scallops with two annual rings averaged 85.8 mm in shell height and ranged between 65 and 99 mm. Of the 93 scallops having 3 annual rings from the Kodiak area, 81 were mature (87%) and 12 were immature. Of the 22 scallops with 3 annuals rings from the Yakutat area, 13 were mature (59%) and 9 were immature. All 9 scallops with 2 annual rings were immature and all scallops with 4 or more annual rings were mature (Table 3).

The majority of scallops reach sexual maturity after formation of the third annual ring and all are mature after formation of the fourth ring. Unfortunately, the sample size of scallops having four or fewer annual rings present was small as a result of their scarcity in commercial catches. Scallops with three annual rings from Kodiak averaged 19 mm larger than those from Yakutat, and the difference for scallops with four annual rings averaged 23 mm. It is apparent

TABLE 3. Average size at maturity for weathervane scallops from the Kodiak and Yakutat areas of Alaska, 1968–69.

Area sampled	Sample size	Avg shell height (*mm*)	Size range (*mm*)	No. annual rings	Mature	Immature
Kodiak Is.	71	123.8	98–147	4	71	0
Yakutat	14	100.6	94–115	4	14	0
Kodiak Is.	93	104.2	74–128	3	81	12
Yakutat	22	84.6	73–92	3	13	9
Kodiak Is.	9	85.8	65–99	2	0	9
Totals	209				179	30

that growth is more rapid in the Kodiak area than in the Yakutat area. This difference in average growth rates and size apparently is not related to sexual maturity as the majority of scallops from both areas reached sexual maturity after formation of the third annual ring.

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The Banded Gunnel, *Pholis fasciatus*, in Newfoundland[1,2]

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GREEN, J. M. 1970. The banded gunnel, *Pholis fasciatus*, in Newfoundland. J. Fish. Res. Bd. Canada 27: 2120–2121.

Pholis fasciatus was collected at two Newfoundland sites. These are the most southerly records, as well as the first Newfoundland records, of *P. fasciatus* in the North Atlantic. The meristic counts of the Newfoundland specimens are near or below the low end of the range ordinarily given for the species.

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THE BANDED GUNNEL, *Pholis fasciatus* (Bloch and Schneider), has not previously been reported from Newfoundland. Backus (1957) and Gordon and Backus (1957) reported on two and seven specimens, respectively, collected at several sites on the Labrador coast between Hamilton Inlet (54°20′N) and Saglek Bay (58°30′N). Leim and Scott (1966) list these as the most southerly as well as the only Labrador records of *P. fasciatus* in the North Atlantic.

During the course of studies of Newfoundland littoral fishes commenced in June 1968 by the author and his graduate students, seven specimens of *P. fasciatus* were taken in general fish collections with Chem Fish Collector used as a fish toxicant. On July 4, 1968, six specimens, 23.5–30.3 mm long, were collected approximately 200 m west of the lighthouse on Moores Point near St. Anthony (55°33′N). A single specimen, 21.8 mm long, was taken on July 10, 1968, at Bear Cove, White Bay (49°50′N). Both collections were made at low tide with only skin diving equipment and small dipnets, and with the depth of capture not exceeding 3–4 m.

At both sites the bottom was bed rock partially covered with loose boulders and stones. Except for a small amount of *Fucus* in the shallowest water, the Bear Cove site was devoid of conspicuous algal cover. The site at Moores Point had extensive algal cover provided mainly by *Alaria* and *Laminaria*. Of the two sites, Moores Point was the more exposed. Water temperatures at Moores Point and Bear Cove were 5 and 10 C, respectively.

The meristic counts (Table 1), made from X-ray negatives, fall within or slightly below the ranges given by Jensen (1942) and Leim and Scott (1966). The counts are all at the lower end of the ranges or below, whereas those given by Gordon and Backus (1957) for dorsal fin spines from Labrador specimens are near the upper end of the range. Probably there is a clinal variation

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TABLE 1. Total length and meristic counts of *Pholis fasciatus* from Moores Point (first six) and Bear Cove (last), Nfld. Ranges given by Leim and Scott (1966) are in parentheses, those by Jensen (1942) are marked with an asterisk (*).

Length (*mm*)	Dorsal fin spines (84–91)*	Anal fin rays (43–46)*	Vertebrae 91–98*
29.6	84	43	90
29.4	85	45	90
29.0	85	45	90
29.3	83	44	89
30.3	85	45	91
23.5	85	45	91
21.8	84	46	90

in meristic characteristics in *P. fasciatus*, correlated with temperature, as has been described by Jensen (1942) in *Pholis gunnellus*. Complete pelvic fins were present on all specimens.

The color patterns, especially the dorsal spots and lateral banding, were very similar to those described by Jensen (1942). The sides of these specimens were not brilliantly scarlet as noted by Leim and Scott (1966), but *P. fasciatus* were easily distinguished from *P. gunnellus* taken in the same collections, 15 at Moores Point and 35 at Bear Cove, on the basis of differences in the dorsal spots and the color pattern of the head and sides.

All specimens of *P. fasciatus* have been deposited in the Memorial University Ichthyological Collection.

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